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Interactions of myelin basic protein with palmitoyllysophosphatidylcholine: characterization of the complexes and conformations of the protein

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Abstract. The stoichiometry of palmitoyllysophosphatidylcholine/myelin basic protein (PLPC/MBP) complexes, the location of the protein in the lysolipid micelles, and the conformational changes occurring in the basic protein and peptides derived from it upon interaction with lysolecithin micelles were investigated by circular dichroic spectropolarimetry, ultracentrifugation, electron paramagnetic resonance (EPR) and ^{31}P, ^{13}C, and ^{1}H nuclear magnetic resonance spectroscopy (NMR), and electron microscopy. Ultracentrifugation measurements indicated that well-defined complexes were formed by the association of one protein molecule with approximately 141 lysolipid molecules. Small-angle X-ray scattering data indicated that the PLPC/MBP complexes form particles with a radius of gyration of 3.8 nm. EPR spectral parameters of the spin labels 5-, and 16-doxylstearate incorporated into lysolecithin/basic protein aggregates, and ^{13}C- and ^{1}H-NMR relaxation times of PLPC indicated that the addition of the protein did not affect the environment and location of the labels and the organization of the lysolipid micelles. The data suggested that MBP lies primarily near the surface of the micelles, with segments penetrating beyond the interfacial region into the hydrophobic interior, but without any part of the protein being protected against rapid exchange of its amide groups with the aqueous environment. The basic protein acquired about 20% α-helix when bound to lysolipid micelles. Circular dichroic spectra of sequential peptides derived by cleavage of the protein revealed the formation of α-helical regions in the association with lysolecithin. Specific residues in myelin basic protein that participated in binding to the micelles were identified from magnetic resonance data on changes in the chemical shifts and intensities of assigned resonances, and line broadening of peaks by fatty acid spin-labels incorporated into the micelles.

Key words: Myelin basic protein – Palmitoyllysophosphatidylcholine – NMR – EPR – C D – Ultracentrifugation

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Introduction

Understanding the structure and physicochemical properties of myelin has provided the rationale for investigations of the interactions of myelin basic protein (MBP) with lipids, lysolipids and detergents in model systems (Boggs et al. 1986; Mendz et al. 1984; Nicot et al. 1985; Chatenay et al. 1985; Mendz et al. 1988, 1990, 1991, 1992; Nicot et al. 1993). Many studies naturally have focused on the associations of MBP with phospholipids because they constitute the largest lipid component of the myelin membrane. The important problem of determining the role of the basic protein in stabilizing the double bilayer structure of myelin required investigations of its interactions with different classes of phospholipids (Surewicz et al. 1985, 1987; Sankaram et al. 1989), and with phosphatidylcholines in particular, owing to their abundance in myelin. The evidence for hydrophobic interactions of MBP with lipids is mostly indirect, relying on observations of changes in the average properties of whole lipid populations. Reports on the associations of the protein with lecithins are not in complete agreement, the interactions with these neutral phospholipids appear to depend on the experimental conditions and on the techniques employed in the measurements (Keniry and Smith 1979; Young et al. 1982; Cheifetz and Moscarello 1985). Little information was obtained from these model systems regarding the forces and nature of the binding, the relative locations of protein and lipid molecules, and the conformations adopted by the protein bound to lipid structures.

More conclusive results were derived from NMR studies of the binding of myelin basic to lysophosphatidylcholines (Hughes et al. 1982; Smith 1982). Nonetheless, the observed changes in the ^{1}H-NMR spectra of the basic protein could not be interpreted simply as evidence for contacts between protein residues and the hydrocarbon chains because they may have arisen from protein-protein interactions promoted by the lysophospholipids. Data on the interactions of MBP with the detergent dodecylphosphocholine suggested that the polypeptide backbone is mainly outside the surface of the micelles, with only spe-

cific regions on the protein molecule penetrating into the micelle core (Mendz et al. 1988). Studies of the binding of the protein to mixtures of neutral and charged detergents suggested that a balance between hydrophobic and ionic forces is achieved in the interactions of the protein with mixed detergent micelles (Mendz et al. 1990).

Investigations of the interactions of myelin basic protein with lysophosphatidylcholines of acyl chain lengths from 6 to 18 carbon atoms indicated that the conformational changes induced in the protein molecule depended on the acyl chain length of the lysolipid in the suspensions, the maximum α-helicity was induced in the presence of palmitoyllysophosphatidylcholine (Mendz et al. 1992). Among the protein segments with high helix-forming probability the data suggested that three regions of the protein molecule may be involved in the binding to lysolecithins of different acyl chain lengths (Mendz et al. 1992). However, to account for the number of residues in helical conformations in MBP bound to palmitoyllysophosphatidylcholine, it would be necessary that residues from at least one other region also participate in the protein-lysolipid interactions.

In the present work physicochemical properties of palmitoyllysophosphatidylcholine/myelin basic protein complexes have been characterized and specific regions of the polypeptide chain that form α-helix in the presence of the lysophospholipid have been identified.

Materials and methods

Experimental

Palmitoyllysophosphatidylcholine (PLPC) and [$^2H_{31}$] PLPC with the acyl chain protons substituted by deuterium atoms, were obtained from Serdary Research Laboratories Inc. (London, Ontario). The lysolipids were employed without any further purification. Rabbit and bovine myelin basic proteins (r-MBP, b-MBP) were prepared and purified according to the method of Law et al. (1984). Porcine MPB (p- MBP) was kindly provided by Dr Max Marsh of Eli Lilly Co. Peptides were prepared and purified from r-MBP by cleavage with porcine pepsin (EC 3.4.23.1) or human thrombin (EC 3.4.21.5) and purified as previously described (Law et al. 1984; Martenson et al. 1981a, 1981b). The fatty acid spin-labels 5-doxylstearate [2-(3-carboxypropyl)-2-tridecyl-4,4-dimethyl-3-oxazolidinyloxy] and 16-doxylstearate were purchased from Aldrich Chemical Co. (Milwaukee, WI). All other reagents were of analytical grade. The numbering of residues in the protein sequence is the same as employed in Fig. 8 of Mendz et al. (1984). This convention was adopted to maximize the homologies in the primary structure of proteins derived from different species.

Circular dichroism

Circular dichroic (CD) spectra were measured at 295 K with a JEOL-500C spectropolarimeter at various lysolipid/protein ratios. Spectral range was 195–275 nm, and 4–16 spectra were accumulated over 8K data points. Typical instrumental conditions were as follows: sensitivity 1 (m° deg)/cm, scanning speed 10 nm/min, and time constant 4 s. Samples were prepared in quartz cells with path lengths from 0.1 to 1.0 mm. Bovine MBP concentrations were 1 to 12×10^{-6} M, and the concentrations of peptides 2.5 to 3.3×10^{-5} M; PLPC concentrations ranged from 1×10^{-6} to 6.7×10^{-3} M. Measurements were carried out in 0.01 M phosphate buffer, pH 7.0. The CD spectrum of lysolecithin alone was negligible over the spectral range recorded. The mixed protein/lysolipid suspensions were optically clear in the range of concentrations employed. The α-helix content of the protein and peptides were calculculated by fitting the CD spectrum to a weighted average of values of α-helical, random-coil, and β-sheet spectra according to the method of Keniry (1981). Absolute values of α-helicity are uncertain to about ±4%, but comparative values are reproducible to ±0.5%. The absolute values of the calculated β-sheet content of the spectra are uncertain to about ±15%, and the errors in the values determined for nonordered structures are considered larger than ±15%.

Ultracentrifugation

Measurements were carried out in 0.05 M phosphate buffer, pH 7.0, with 10×10^{-3} M PLPC, and 5×10^{-5} M r-MBP. Lysolecithin suspensions in buffer similar to the ones in the sample compartments were placed in the reference compartment of the cell. Sedimentation equilibrium experiments were performed in a Beckman Model E analytical ultracentrifuge at 293 K. Samples were spun in a Four-Place An-F rotor with double-sector cells at 20000 rpm for typically 24 h. After the mixtures reached equilibrium, the MBP concentration was monitored with ultraviolet light at 280 nm. The stoichiometry of the lysolipid/protein complex was determined by the method of Reynolds and Tanford (1976).

Electron paramagnetic resonance

Samples containing 2×10^{-3} M spin-labels and PLPC at concentrations ranging from 5 to 180×10^{-3} M, with or without p-MBP at a lysolipid/protein molar ratio 180:1, were prepared in 0.05 M ^2H_2O phosphate buffer, pH 7.0, and placed in capillary tubes. EPR measurements were carried out at 295 K with a Varian E4 X-band spectrometer and spectra were recorded over 2 K data points, using lock-in detection with magnetic field modulation at 100 kHz.

The lysolipid/spin-label concentration ratios at which the peak-to-peak width of the $I=-1$ transition of the labels became constant were taken to indicate that no interactions occurred between the doxylstearate molecules bound to the same lysolecithin micelle. It was not possible to measure the linewidth directly for all the spectra obtained in this study owing to the excessive broadening of the signals at low lysolipid/spin-label molar ratios and the anisotropic nature of the spectra of the 5-doxylstearate label. Linewidths were determined indirectly by numerically in-

tegrating twice the first derivative spectra to obtain the area A under the absorption curve. This area is given by

$$A = L\,h\,(\Delta\nu)^2 \quad (1)$$

where L is the lineshape factor, h is the height of the first derivative spectrum and $\Delta\nu$ is the peak-to-peak width. In the spectra measured in this work the ratios of the heights of the constituent lines remained approximately the same. Using a lineshape factor equal to unity and taking h as the height of the $I=0$ transition, a measure of the peak-to-peak width in arbitrary units was obtained from the relationship

$$\Delta\nu = \sqrt{A/h}. \quad (2)$$

Rotational correlation times, τ_r, for the 16-doxylstearate spin-label were calculated from the spectra by (Freed 1976; Marsh 1981)

$$\tau_r = (6.5 \times 10^{-10})\,\Delta H\,\{[I(0)/I(-1)]^{1/2} + [I(0)/I(-1)]^{1/2} - 2\} \quad (3)$$

where $I(0)$, $I(+1)$, and $I(-1)$ are the peak-to-peak heights of the 0, +1, and −1 transitions in the first-derivative spectrum, and ΔH is the linewidth (in gauss) of the 0 transition.

For the 5-doxylstearate spin-label the order parameter S was calculated from (Marsh 1981)

$$S = A_{ZZ}/A_{ZZ}^{R}; \quad (4)$$

where A_{ZZ} is the distance between the outer peaks of the spectrum and A_{ZZ}^{R} is the corresponding quantity in the rigid-limit. The value of the latter parameter (in gauss) was calculated from the expression (Marsh 1981)

$$A_{ZZ}^{R} = 2.35\,a_{14N} - 0.84$$

where the ^{14}N hyperfine splitting is $a_{14N} = 1/3(2A_{\perp} + A_{\prod})$, with $A_{\perp}$ and $A_{\prod}$ the separation between the inner and outer lines of the anisotropic spectrum, respectively (Freed 1976; Marsh 1981). A correction to the value of $A_{\perp}$ measurement directly from the peak positions is sometimes required (Marsh 1981), but it was negligible in the present case.

Small-angle X-ray scattering (SAXS)

Lysolipid solutions at concentrations of 160×10^{-3} M were prepared in 0.05 M phosphate buffer, pH 7.0. Suspensions including the protein were prepared by adding 1×10^{-3} M porcine MBP. the samples were allowed to allowed to equilibrate for over 24 h. Measurements were carried out using a high resolution SAXS camera (Aldissi et al. 1988). The instrument employs 0.15 nm X-rays, has a 1 m focal length, and wavelength selection and focusing of X-rays is achieved by a bent quartz crystal. Owing to the very low contrast between the detergent micelles and the background water, 0.03×10^{11} cm^{-2}, desired higher intensities of the beam were obtained by not employing focusing in the vertical direction (using a full Huxley-Holmes geometry) which was not needed for these experiments. The small-angle scattering patterns were recorded on a one-dimensional position sensitive detector (Gabriel 1977). Data were processed using the ORL-ANU programme suite and all the scattering patterns were recorded on an absolute intensity scale calibrated using a 1 mm water cell. Scattering length densities were calculated in the usual way (Jacrot 1976). Intensities were measured as functions of the momentum transfer Q, with

$$Q = (4\,\pi \sin\theta)/\lambda \quad (5)$$

where θ is the half-scattering angle and λ the wavelength of the X-rays.

^{13}C- and ^{31}P-nuclear magnetic resonance

^{13}C-NMR spectra at 125.7 MHz were acquired on a Bruker AM-500 spectrometer operating in the pulsed Fourier transform mode with quadrature detection. Spectra of PLPC suspensions with or without MBP were measured at 300 K over 32K data points; acquiring 720 transients with radiofrequency pulses of 70° (3 μs), spectral widths of 8064 Hz, and a repetition time of 2.1 s. Free induction decays were transformed employing a line broadening of 1 Hz. Relaxation times of the ^{13}C resonances of protonated carbon atoms and the ^{31}P resonance of the phosphorus atom of the phosphorylcholine headgroup of PLPC were measured in lysolecithin suspensions with or without MBP on a Varian XL-400 spectrometer at 300 K. ^{13}C relaxation spectra at 100.5 MHz were obtained by averaging 256 transients over 16K data points per spectrum, with spectral width of 6920 Hz, radiofrequency pulses of 90° (22 μs), and a repetition time of 20 s. Free induction decays were transformed employing a linebraodening of 3 Hz. ^{31}P-NMR relaxation spectra at 162 MHz were acquired by averaging 56 transients over 16K data points. A radiofrequency pulse of 90° (31.1 μs) was used, with a spectral width of 3065 Hz and a repetition time of 22 s. Free induction decays were transformed with a linebroadening of 1 Hz. Longitudinal relaxation times (T_1) were measured by the inversion-recovery pulse sequence (Vold et al. 1986), with delay times of 0.001–15 s for ^{13}C resonances and 0.001–20 s for ^{31}P resonances. Transverse relaxation times (T_2) were measured with the spin-echo pulse sequence (Carr and Purcell 1954), with delay times of 0.004–0.12 s for ^{13}C resonances, and 0.004–0.86 s for ^{31}P resonances. In the relaxation experiments arrays of 18 spectra were collected with interleaved acquisitions for each experiment. T_1 and T_2 values were calculated by non-linear regression analysis (Miller 1981). The assignments of ^{13}C resonances are those of Levine et al. (1972); chemical shifts are given relative to 3-trimethylsilyl [2,2,3,3-$^{2}H_4$] propionate at p^{2}H 7.0.

^{1}H-nuclear magnetic resonance

Polypeptide and polypeptide/lysolipid suspensions were lyophilized twice from 99.5% $^{2}H_2O$ (Australian Institute for Nuclear Science and Engineering, Lucas Heights, NSW) and dissolved in 99.96% $^{2}H_2O$ (Merck Sharp & Dohme) at polypeptide concentrations 0.1 to 1.0×10^{-3} M in 5-mm OD precision NMR tubes (Wildmad, Buena, NJ). The pH was adjusted with ^{2}HCl or $NaO^{2}H$ and measured

with an Activon BJ331 combination glass thin electrode, values being reported as meter readings. Increasing amounts of lysolecithin were added to protein or peptide solutions to obtain a series of lysolipid/polypeptide molar ratios from 0 to 150. ^{1}H-NMR spectra at 400 Mhz were measured on a Bruker WM-400 spectrometer, and at 500 MHz on a Bruker AM-500 spectrometer, operating in the pulsed Fourier transfrom mode with quadrature detection. ^{1}H-NMR spectra of the polypeptides were measured at 298 K over 16K data points; acquiring 1000–2000 transients with radiofrequency pulses of 70° (4–6.5 μs), spectral widths of 4000–5000 Hz, and a repetition time of 2.1 s. To avoid dynamic range problems in measuring these spectra, the lysolecithin resonances corresponding to glycerol backbone and choline headgroup protons were presaturated for 0.03 s. Spectra were transformed by employing either no window functions or Gaussian multiplication with line broadenings of -1 to -3 Hz and a Gaussian broadening factor of 0.19. Chemical shifts were measured relative to internal DSS (sodium 4,4-dimethyl-silapentane-1-sulfonate) by the method previously described (Mendz et al. 1982). Relaxation measurements of ^{1}H resonances of PLPC in suspensions with or without MBP, were performed at 300 K by averaging 8 transients over 16K data points per spectrum, with spectral width of 5000 Hz, radiofrequency pulses of 90° (12.5 μs), and a repetition time of 20 s. Free induction decays were transformed employing a linebroadening of 0.5 Hz. Longitudinal relaxation times (T_1) were measured by the inversion-recovery pulse sequence (Vold et al. 1968), with delay times of 0.01–20 s. T_1 values were calculated by non-linear regression analysis (Miller 1981).

The exchange with ^2H_2O of -NH and -NH_2 groups in the protein/lysolipid complexes was followed by lyophilizing samples from H_2O, resuspending the mixtures in ^2H_2O, and measuring the NMR spectra every 10 min.

Suspensions containing spin-labels were prepared by adding a measured amount of free radical in ethanol to PLPC/MBP aqueous suspensions to obtain the final composition desired, lyophilizing the mixture, and dissolving it in ^2H_2O.

Results

Circular dichroism

The CD spectra of b-MBP and sequential peptides derived from r-MBP were measured in phosphate buffer with increasing concentrations of PLPC, up to lysolipid/polypeptide molar ratios of 210:1. Conformational changes occurred in the polypeptides interacting with the lysolecithin (Fig. 1). The helicity induced in the basic protein molecules was a function of the lysolipid/protein molar ratio (Fig. 2). At PLPC concentrations below the critical micelle concentration (cmc) little conformational changes occurred in the protein, but the α-helix content of MBP increased rapidly when the concentration of lysolipid in the suspension reached the cmc. Changes in the basic protein CD spectrum were complete only at PLPC/MBP molar ratios about 150:1, suggesting that the stoichiometry of

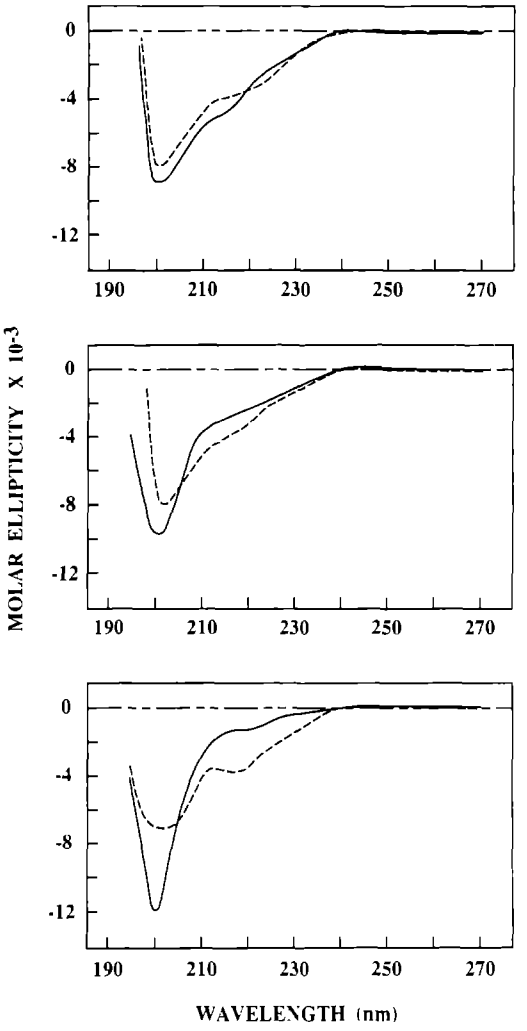

Fig. 1. Circular dichroic spectra of MBP and sequential peptides in 150:1 lysolipid/polypeptide mixtures at 295 K in 0.05 M phosphate buffer, pH 7. *Top:* peptides 1–90 (——) and 91–179 (- - - -). *Middle:* peptides 1–98 (——) and 99–179 (- - - -). *Bottom:* peptide 1–179 (myelin basic protein) in aqueous solution (——) and in detergent suspension (- - - -)

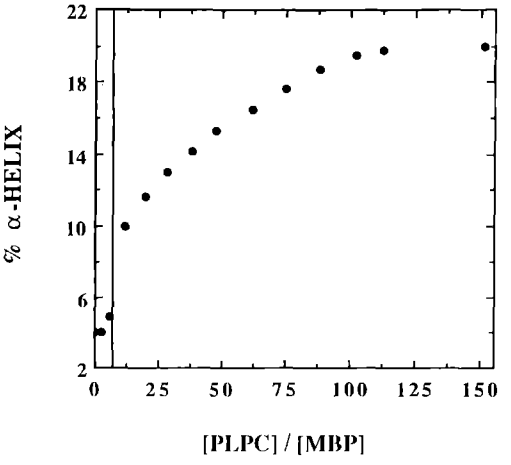

Fig. 2. Calculated percent α-helix of bovine myelin basic protein as a function of lysolipid/protein ratio at 295 K, in 0.05 M phosphate buffer, pH 7. The vertical line at a PLPC/MBP ratio of 9 indicates the cmc of PLPC, at approximately 10×10^{-6} M

Table 1. Mole percent of α-helix and number of residues in helices of bovine MBP and sequential peptides in aqueous solution and PLCP suspensions at 295 K and pH 7 in 0.05 M phosphate buffer

Peptide	mol % of α-helix		α-helical residues	
	H_2O[a]	PLPC	H_2O[a]	PLPC
1–90	8.6	16.7	8	15
91–179	4.0	10.9	3	9
1–98	11.8	21.3	11	20
99–179	4.4	17.7	3	12
1–179[b]	4.0	20.0	7	34

[a] From Mendz et al. (1984)
[b] From Mendz et al. (1992)

the complex was 1 protein molecule per 150 lysolipid molecules. These results ensured that under the conditions of the NMR experiments described below, essentially all MBP is bound to micelles and each protein molecule has a full complement of lysolipid. The calculated mole percentage α-helix induced in myelin basic protein and sequential peptides derived from r-MBP in palmitoyllsophosphatidylcholine suspensions at maximal α-helicity are given in Table 1; for comparison the values previously obtained for aqueous solutions are also given in the table.

Ultracentrifugation

Ultracentrifugation was used to determine the stoichiometry of PLPC/MBP aggregates and to obtain information on their homogeneity. The state of aggregation of MBP bound to PLPC micelles was determined by equilibrium centrifugation in $H_2O/^2H_2O$ mixtures (Reynolds and Tanford 1976). At a solvent density that matches the density of the lysolecithin, the apparent molecular weight of the complex is determined by the molecular weight of the protein component. A plot of the apparent value $M_p(1\text{-}\phi'\rho)$ as a function of solvent density for solutions with a lysolipid/protein molar ratio of 200:1 is shown in Fig. 3 (top). The arrow in this figure indicates the solvent density, 1.05374 $g \cdot cm^{-3}$ (Haberland and Reynolds 1975), at which the apparent molecular weight of the complex is that of the MBP monomer. The slope of the plot in Fig. 3 (top) yields a relatively inaccurate estimate of 141 ± 20 PLPC molecules per protein molecule in the complex. Thus the particle weight of the lysolecithin/basic protein complex was 90800 ± 9800. Since these calculations assumed the homogeneity of the PLPC/MBP aggregates, it was necessary to establish the validity of this hypothesis. Nichol and co-workers (Milthorpe 1975) introduced a function $\Omega(r)$ of the activity of the monomer at different positions of the radial distance, r, in the ultracentrifugation cell, as a means of fitting reaction models to sedimentation equilibrium data obtained with self-associating solutes. The $\Omega(r)$ function provides a very sensitive test to determine the homogeneity of a sample. Figure 3 (bottom) shows a plot of experimentally determined values of Ω as a function of the protein concentration in the cell, $c(r)$, for one of the ultracentrifugation runs. The value 1 for $\Omega(r)$ indicated a homogeneous distribution of the protein in the sample.

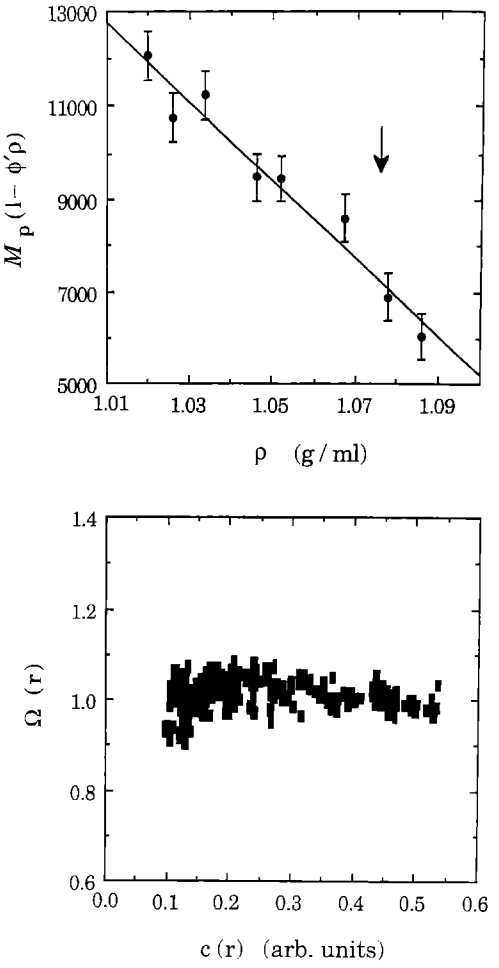

Fig. 3. *Top:* Sedimentation equilibrium measurements by the method of Reynolds and Tanford (1976) for a mixture of 10×10^{-3} M PLPC and 5×10^{-5} M rabbit MBP in 0.05 M phosphate buffer, pH 7.0 at 293 K. The apparent molecular weight $M_p(1\text{-}\phi'\rho)$ is plotted versus the solvent density ρ, which was altered by mixing H_2O and ^2H_2O in various proportions. M_p is the molecular weight of the protein component of the aggregate, and $\phi'\rho$ is the volume increment per gram of protein. The arrow indicates the value at which the apparent weight of the aggregate corresponds to that of the protein. *Bottom:* Plot of the experimentally determined values of the dimensionless function $\Omega(r)$ versus the total protein concentration in the cell

EPR measurements of spin-labels bound to PLPC/MBP micelles

Spectra of 2×10^{-3} M 5-doxylstearate and 16-doxylstearate spin-labels measured in lysolecithin and lysolecithin/protein suspensions are shown in Fig. 4. Three lines were recorded in the first derivative spectra corresponding to the I=+1, 0 and –1 electronic transitions. The lysolipid/spin-label molar ratios in the samples decreased from the top row to the bottom row of spectra in the figure. At high relative lysolipid concentrations (top row) the spectra arising from label molecules in the micelles were rea-

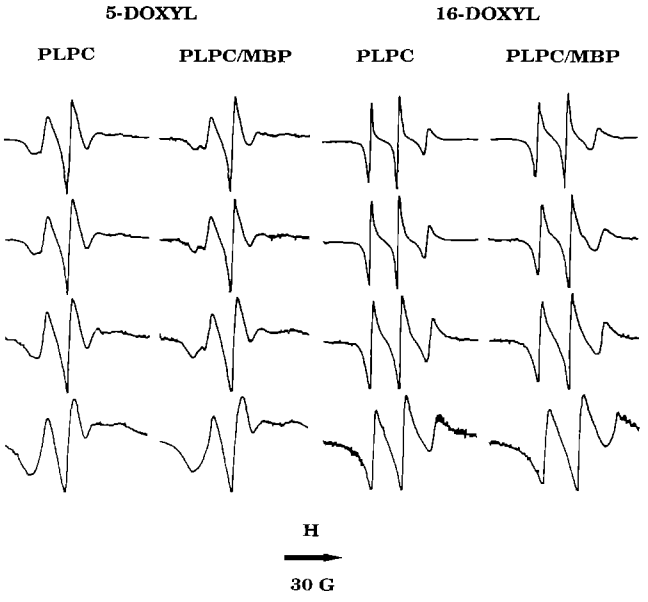

Fig. 4. X-band EPR spectra for 2×10^{-3} M 5- and 16-doxylstearate labels as a function of PLPC and PLPC/MBP concentrations. Spectra were measured in 0.05 M phosphate buffer at pH 7.0 and 295 K. *From top to bottom:* PLPC concentrations were 250×10^{-3} M, 120×10^{-3} M, and 10×10^{-3} M. In the aggregates the PLPC/MBP molar ratio was 180:1

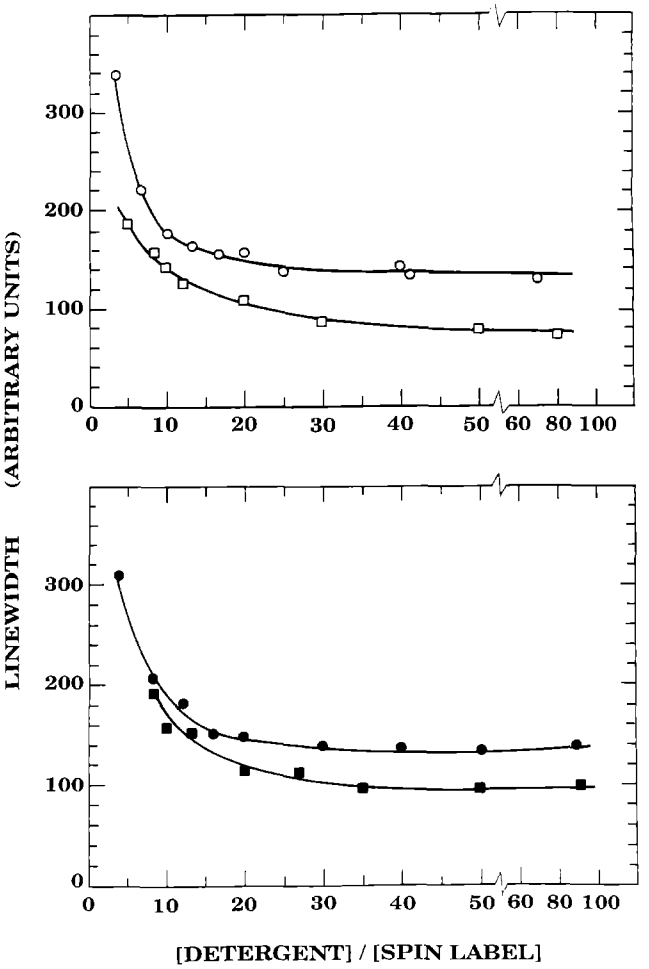

Fig. 5. Peak-to-peak linewidth in arbitrary units for the I=–1 transition in the X-band spectra of the 5-doxylstearate (○, ●) and 16-doxylstearate (□, ■) spin-labels as a function of the ratio of detergent to label concentrations. Spectra were measured in 0.05 M phosphate buffer pH 7 and 295 K. The top panel (*open symbols*) corresponds to PLPC micelles and the bottom panel (*full symbols*) to PLPC/MBP complexes at molar ratio 180:1

sonably sharp, indicating that there was no significant interaction between spin-label molecules bound to the same micelle. As the detergent concentration was decreased relative to the label cencentration, micelles were formed that contained interacting spin-label molecules; these interactions resulted in broadening of the spectral lines. The variation of the peak-to-peak linewidth Δv in the EPR spectra of the 5- and 16-doxylstearate labels as a function of the lysolipid/spin-label ratio in PLPC and PLPC/MBP suspensions are plotted in Fig. 5. For the bound labels the linewidths of this transition decreased sharply as the lysolecithin/label ratio was increased from 5:1 to approximately 50:1. For both labels the linewidths became independent of the detergent/spin-label ratio at approximately 50 detergent molecules per spin-label, indicating that at this ratio the label molecules bound to a micelle did not interact. Palmitoyllysophosphatidylcholine micelles can occur in two different sizes depending on the concentration of lysolipid. In suspensions at concentrations below 5×10^{-5} M small micelles are formed with approximately 60–70 monomers per micelle, above this concentration the micelle size becomes of the order of 150 monomers (Haberland and Reynolds 1975). Lysolecithin suspensions at milimolar concentrations were employed for the EPR measurements, thus the lysolipid would form the larger micelles. The data from PLPC suspensions indicated that there could be up to three spin-label molecules per micelle without significant interactions between them. The results suggested that the addition of MBP did not induce any significant change in the size of the PLPC micelles.

^{14}N hyperfine splittings, a_{14N}, rotational correlation times, τ_r, and order parameters, S, provided further information about the organization of the PLPC/MBP complexes. Comparison of the a_{14N} values for free spin-label, PLPC micelles, and PLPC/MBP aggregates showed decreases in the hyperfine splitting of labels incorporated into lysolipid micelles with or without protein (Table 2). The decrease in a_{14N} indicated a less polar environment for the nitroxide moiety of the bound spin-label (Griffith and Jost 1976). The comparable values of the splitting in the presence of lysolipid with or without protein indicated that the local environment of the label is largely unaffected by the presence of MBP. The rotational correlation times of the 16-doxylstearate spin-labels incorporated into lysolipid micelles increased 12-fold (Table 2), indicating that the rotational mobility of the nitroxide group was reduced compared to its mobility in the free spin-labels, and the corresponding increases in the correlation times for labels incorporated into PLPC/MBP aggregates were 16-fold (Table 2). The order parameter S was 0.71 for 5-doxylstearate incorporated into the lysolipid/protein aggregates. The larger increase in the correlation time and the lower

Table 2. Isotropic hyperfine constants (a_{14N}), rotational correlation times (τ_r) and order parameters S measured for free spin-labels, and for spin-labels incorporated into palmitoyllysophosphatidylcholine micelles and palmitoyllysophosphatidylcholine/myelin basic protein complexes. Samples were in 0.05 M ^2H_2O phosphate buffer, pH 7.0, and placed in capillary tubes; measurements were carried out at 295 K

	1×10^{-4} M spin-label[a]		2×10^{-3} M spin-label 3×10^{-1} M lysolipid			2×10^{-3} M spin-label 3×10^{-1} M lysolipid 1.66×10^{-3} M protein		
	a_{14N} (G)	$10^{10}\tau_r$ (s)	a_{14N} (G)	S	$10^{10}\tau_r$ (s)	a_{14N} (G)	S	$10^{10}\tau_r$ (s)
5-doxylstearate	15.7	1.3	13.7	0.71	–	13.6	0.68	–
16-doxylstearate	15.7	0.82	13.8	–	9.5	13.6	–	13

[a] From Brown et al. (1981)

value of the order parameter of the labels measured in the lysolecithin/basic protein complexes may indicate a further minor restriction in their rotational mobility.

Small-angle X-ray scattering

The scattering spectra of palmitoyllysophosphatidylcholine micelles and complexes with p-MBP showed large, broad peaks which in all probability correspond to difraction peaks, since there are second very broad features at twice the Q values. The results indicated that at these concentrations, the aggregates are close enough to give an interference pattern, suggesting a certain degree of ordering in the suspensions and/or the presence of larger aggregates. The spectra of PLPC micelles and PLPC/MBP aggregates were analyzed using the Guinier approximation, and yielded a radii of gyration of 3.5 ± 0.5 and 3.8 ± 0.4 nm, respectively.

^1H, ^{13}C and ^{31}P NMR relaxation times of PLPC

To avoid possible effects of paramagnetic ions on the relaxation times of the different nuclei, EDTA (1×10^{-4} M) was added to samples. Similar values were measured for the spin-lattice relaxation times (T_1) of the carbon nuclei of PLPC in micelles with or without myelin basic protein (Table 3). Small differences in these values were observed only for carbon nuclei in positions 2 and 3 of the acyl chain, with position 1 corresponding to the methyl group of the chain. The formation of lysolipid/protein aggregates resulted in a general broadening of ^{13}C spectral lines and, with the exception of carbon nuclei at positions 1 and 14, no significant changes were measured for the spin-spin relaxation times (T_2) of PLPC backbone and headgroup carbon nuclei (Table 3). Addition of chloroform (43 µl/ml) to lysolecithin or lysolecithin/basic protein suspensions did not affect the relaxation times of the glycerol and phosphoryl choline moieties.

The ^{31}P spin-lattice and spin-spin relaxation times of the phosphoryl group of PLPC increased in lysolecithin/protein complexes relative to those for lysolipid micelles (Table 3). The faster motions observed for the phosphorus nuclei suggested that the presence of the protein

Table 3. ^{13}C and ^{31}P transverse (T_2) and longitudinal (T_1) relaxation times of protonated carbon[a] and phosphorus nuclei of 60×10^{-3} M palmitoyllysophosphatidylcholine suspensions with and without 0.5×10^{-3} M myelin basic protein in 0.05 M phosphate buffer, 5×10^{-4} M EDTA at pH 7 and 310 K

Nucleus	T_2 (ms)		T_1 (ms)	
	PLPC	PLPC/MBP	PLP	PLPC/MBP
C_1[b]	32 ± 4	11 ± 2	2803 ± 350	2865 ± 345
C_2	31 ± 4	20 ± 2	1308 ± 300	1251 ± 150
C_3	37 ± 4	22 ± 3	1045 ± 290	985 ± 120
C_{4-13}	33–37 ± 4	19–22 ± 3	536–781 ± 94	506–601 ± 70
C_{14}	32 ± 4	16 ± 2	462 ± 55	465 ± 55
C_{15}	30 ± 3	27 ± 3	573 ± 69	535 ± 65
C_{G1}[c]	32 ± 4	28 ± 3	191 ± 23	177 ± 21
C_{G2}	34 ± 4	25 ± 3	345 ± 41	335 ± 40
C_{G3}	32 ± 4	27 ± 3	207 ± 25	216 ± 26
C_{C1}[d]	30 ± 4	25 ± 2	512 ± 61	495 ± 60
C_{C2}	27 ± 3	22 ± 2	439 ± 53	440 ± 53
C_{C3}	21 ± 2	17 ± 2	686 ± 82	663 ± 80
P	125 ± 15	224 ± 27	1009 ± 121	4228 ± 507

[a] The values of longitudinal relaxation times of protonated carbons are from Mendz et al. (1992)
[b] The numbering of carbon atoms starts with the methyl group at the end of the acyl chain
[c] C_{Gi} refer to the glycerol backbone carbon atoms, C_{G1} is bonded to the acyl chain, and C_{G3} to the phosphorylcholine headgroup
[d] C_{Ci} refer to the carbon atoms of the choline moiety of the headgroup, C_{C1} is bonded to the phosphate ester and C_{C3} are the trimethyl ammonium carbon atoms

induced rearrangements of the lysolecithin headgroups. Incorporation of chloroform into PLPC micelles of PLPC/MBP complexes did not change the measured ^{31}P relaxation times.

The locations of the nitroxide moieties of 5- and 16-doxylstearate within the lysolipid micelles and the lysolecithin/basic protein complexes were investigated by observing their paramagnetic contributions to the relaxation rates of nuclei in PLPC molecules. Figure 6 shows the ^{13}C-NMR spectra of 0.10 M palmitoyllysophosphatidylcholine in PLPC/spin-label and PLPC/MBP/spin-label mixtures at molar ratios of 100/0.4 and 100/1/0.4, respectively. The presence of 5-doxylstearate in lysolipid suspensions with or without MBP increased the linewidths of

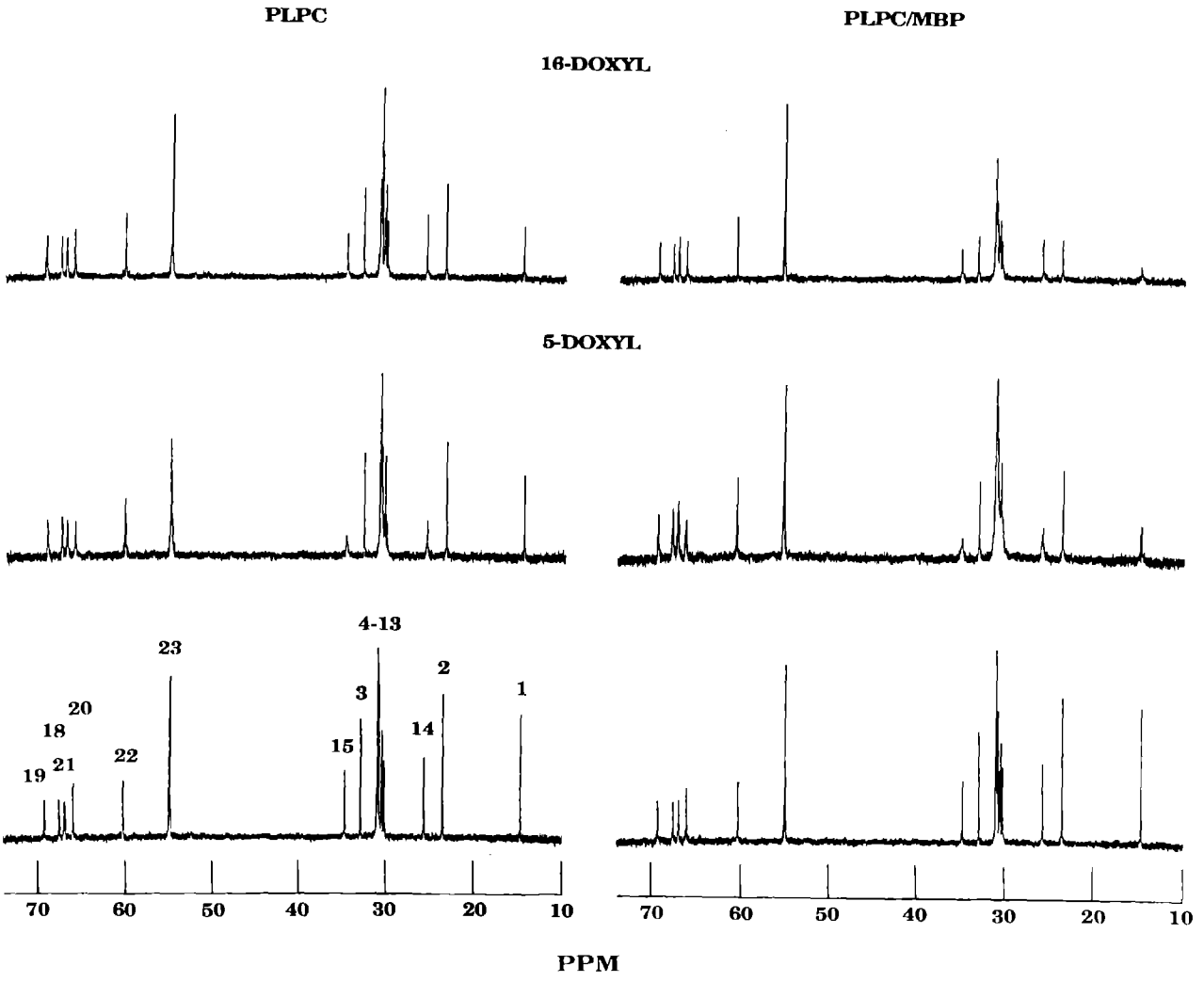

Fig. 6. Effect of incorporation of spin-labels into PLPC micelles (*left*) and PLPC/MBP complexes (*right*). 125.7 MHz ^{13}C-NMR spectra of 0.12 M PLPC suspensions with and without 0.8×10^{-3} M b-MBP, in 0.05 M phosphate buffer, 5×10^{-4} EDTA, pH 7 and 310 K. The spin-label concentration was 1×10^{-3} M. Shown from bottom to top are lysolipid spectra without spin-label, with 5-doxylstearate and with 16-doxylstearate. The resonance assignments to carbon atoms of the PLPC molecule are given on the bottom left-hand side spectrum. The numbering of resonances assigns the following positions to atoms: 1, methyl group of the acyl chain; 2 to 15, acyl methylene groups; 18–20, glycerol backbone atoms; and 21–23 headgroup choline moiety

resonances arising from carbon nuclei 4-13, and the strongest broadening was observed for the resonance of the nucleus in position 15 (Fig. 6). Addition of 16-doxylstearate to lysolecithin suspensions with or without the protein, resulted in increased linewidths in the ^{13}C resonances corresponding to PLPC atoms in positions 1, 2 and 3. The effect was less pronounced in the resonances of successive carbon nuclei of the acyl chain up to position 15 (Fig. 6). Thus, the presence of the spin-labels caused a selective paramagnetic relaxation in the different ^{13}C nuclei of PLPC molecules. The data were qualitatively consistent with an average localization of the nitroxide label of 5-doxylstearate closer to the PLPC micelle surface, and those of 16-doxylstearate near the centre of the lysolipid micelles. The presence of myelin basic protein increased the broadening of the resonances in the hydrophobic core, but the effects of both spin-labels on the ^{13}C resonances of PLPC were qualitively similar with or without the protein, suggesting that the spatial orientation of lysolecithin and of the different spin-labels was not appreciably changed when MBP was also present in the aggregates, although the motions of the acyl chains were affected.

The resonances of the acyl carbons at positions 4–13 partially overlap in the spectra of PLPC; and those of carbons at positions 18–22 show scalar couplings of several hertz to the phosphorus nucleus. As a consequence, measurements of the linewidths of these resonances were less accurate. The broadening effects of the doxylstearate labels were determined more reliably by studying the selectivity of the paramagnetic effects on the ^{1}H spin-lattice relaxation times of PLPC resonances. In the absence of spin-label the proton resonances of PLPC have relaxation times between 535 and 967 ms in micelles, and 509 and 931 ms in lysolipid/protein complexes. The selective increases in linewidth for different protons can be observed in Fig. 7, where the paramagnetic contributions to the relaxation rates ($1/T_1^{P}$) (the difference in measured relaxation rates with and without the spin-label) are plotted as a function

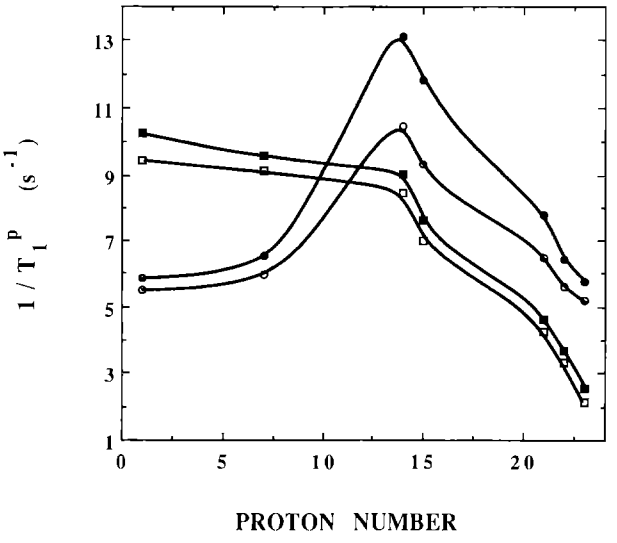

Fig. 7. Paramagnetic contributions of the ^1H spin-lattice relaxation rates, $1/T_1^P$, for the ^1H resonances of PLPC as a function of nucleus position in the covalent structure of the lysolipid molecules. Measurements were carried out at 500 MHz in 10 mM lysolecithin 0.08 mM spin-label suspensions at 300 K. The open symbols represent data for the lysolipid suspensions with 5-doxylstearate (○) or 16-doxylstearate (□), and the full symbols represent data for lysolipid suspensions with 0.075 mM p-MBP with 5-doxylstearate (●) or 16-doxylstearate (■)

of proton number. In suspensions of lysolipid micelles and lysolipid/protein complexes, the largest values of the paramagnetic contribution for the 5-doxylstearate label corresponded to the methylene groups at positions 14 and 15, whereas for the 16-doxylstearate label the largest values of $1/T_1^P$ corresponded to the methyl protons at position 1. These findings are consistent with a location of the nitroxide moiety of the 5-doxylstearate label near the lysolecithin micelle surface, and of the nitroxide group of the 16-label near the end of the acyl chains of PLPC. The addition of MBP caused little changes in the plots of $1/T_1^P$ versus proton position. These observations provided further evidence that the presence of bound protein causes no major change in the location of the nitroxide label within the micelles.

^1H-NMR spectra of polypeptide/lysolecithin complexes

In aqueous solutions at neutral pH myelin basic protein aggregates even at low concentrations (Chapman and Moore 1978; Smith 1985). For this reason the polypeptide spectra reported in these studies were measured at low protein concentration and acidic pH. The use of lysolecithin with deuterated acyl chains allowed observation of the protein resonances in the spectral region between 0 and 3 ppm. However, the protein resonances arising from the glycerol backbone and the choline headgroup of PLPC obscured the protein resonances in the segment 3–5 ppm of the spectrum. Three regions of the ^{1}HNMR spectra of p-MBP in aqueous solutions and in [$^2H_{31}$] PLPC suspensions at a lysolipid/protein molar ratio 150:1, are shown in Fig. 8. Considering that the PLPC/MBP aggregate would have a mass of about to 90 KDa, the protein spectrum remained surprisingly sharp and well resolved in the presence of the lysolipid. Aqueous solutions of b-MBP, p-MBP and the sequential peptides 1–98 and 99–179 of r-MBP were titrated with PLPC up to polypeptide/lysolipid molar ratios of 1:150. Although many of the resonances from the polypeptides were not greatly affected by the

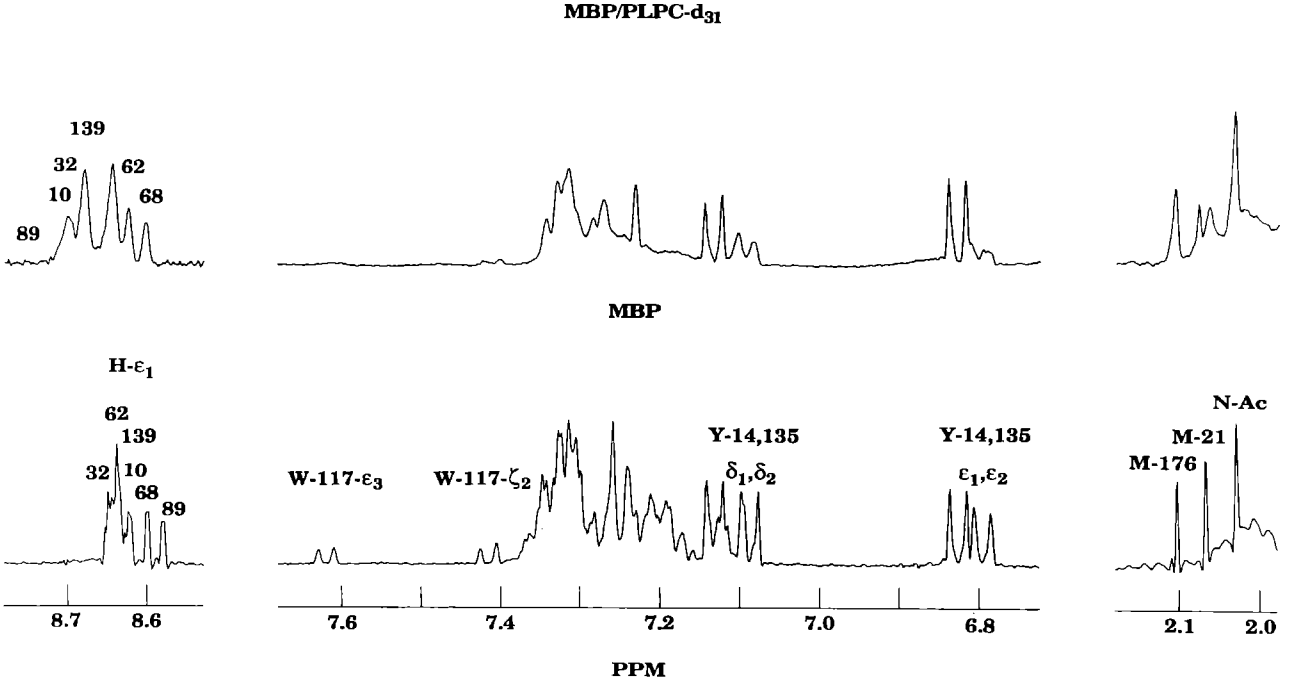

Fig. 8. Three regions of the ^{1}H-NMR spectra of p-MBP in aqueous solution (*bottom*) and in [$^2H_{31}$]PLPC suspension (*top*) at a lysolipid/protein molar ratio 150:1, pH 3 and 298 K. The specific assignments of some resonances and the changes in spectral position of six H-ε_1 resonances are indicated on the figure

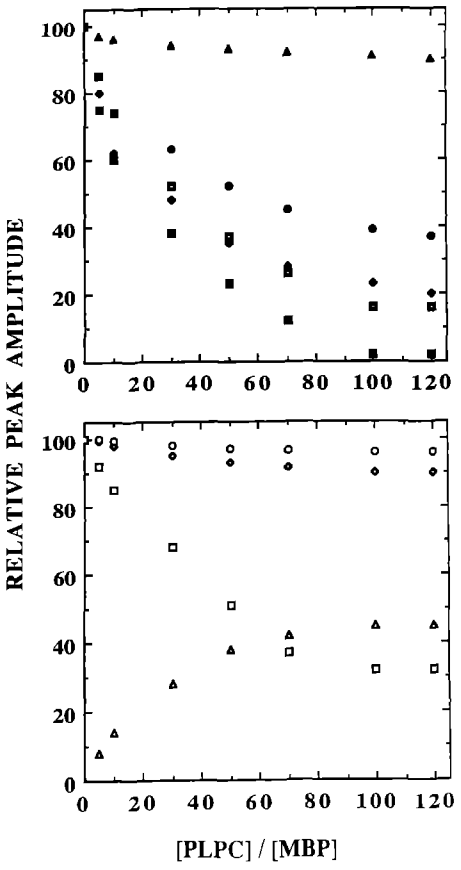

Fig. 9. Broadening of p-MBP resonances as a function of PLPC/MBP molar ratio. *Bottom:* Relative amplitudes of: Met-176-CH_3 (◇), Met-21-CH_3 (△), Met-21-CH_3 (□), and N-Ac-CH_3 (○). *Top:* Relative amplitudes of: Tyr-69-δ-CH_2 (▲), Tyr-14-δ-CH_2 (●), Tyr-135-ε-CH_2 (◆), Tyr-128-ε-CH_2 (◆), Trp-117-ξ_2-CH (■) and Trp-117-ε_3-CH (■)

presence of lysolecithin, selective changes in chemical shifts and/or broadening of resonances were observed, indicating that changes occurred at specific locations of the polypeptides. Owing to the complexity of the ^{1}H-NMR spectrum of MBP, to follow the changes occurring in individual histidyl and tyrosyl residues during lysolecithin titrations it was necessary to perform experiments with the bovine and porcine proteins whose spectra in aqueous solutions show some differences (Mendz et al. 1983; Mendz et al. 1986). The spectral changes were essentially complete at 1:120 protein/lysolipid molar ratios. The effects of titrations on the resonances of the sequential peptides 1–98 and 99–179 were qualitatively similar to those observed for the whole proteins, and the spectral changes induced by the presence of PLPC were complete at 1:70 peptide/lysolipid molar ratios. The titrations with the sequential peptides helped to understand the changes occurring in the protein residues during PLPC titrations.

The changes in the intensities of several p-MBP resonances measured in the presence of the lysolipid are plotted in Fig. 9 as a function of PLPC/MBP concentration. Strong broadening was observed for the peaks arising from ε_1-CH of His-32 and His-89 (Fig. 8); and ε_3-CH and ζ_2-CH of Trp-117 (Fig. 8). Porcine MBP contains four tyrosine residues at positions 14, 69, 128 and 135; broadening occurred in the resonances corresponding to δ-CH_2 of Tyr-14 and Tyr-135, ε-CH_2 of Tyr-14, Tyr-128 and Tyr-135. Small effects were noted on the intensities of the resonances of Tyr-69-ε,δ-CH_2. Of the two methionine residues the resonance of Met-21-ε-CH_3 was strongly broadened and split into two peaks, whereas the linewidth of Met-176-ε-CH_3 resonance showed only a moderate increase. These two residues serve as convenient markers for the N- and C-terminal regions, respectively. The resonances of the methyl moiety of the N-terminal acetyl group, Thr-66 and Thr-67 were slightly broadened by the addition of lysolipid, perhaps reflecting the increase in viscosity of the suspension. The changes observed in the chemical shifts of resonances (Mendz et al. 1992) qualitatively resembled the patterns of the changes in signal intensities. For the histidyl peaks the largest change in chemical shift, 0.140 ppm, occurred for His-89, followed by His-139 and His-10 with changes of 0.111 and 0.050 ppm, respectively (Fig. 8). By contrast, only minor shifts of less than 0.007 ppm were observed in the resonances of histidyls 26, 61, 62, and 68. For the tyrosyl resonances the largest chemical shift change, 0.014 ppm, was observed for Tyr-135, followed by Tyr-128, with a change of 0.013 ppm. Small changes of about 0.008 ppm were noted on the spectral positions of the aromatic resonances of Tyr-14 and Tyr-69. The spectral position of the CH_3 resonance of the N-terminal acetyl group did not change after addition of lysolipid. The shifts observed in the methyl resonances of Thr-66 and -67 were less than 0.003 ppm.

Two regions of spectrum of MBP in [$^2H_{31}$]PLPC suspensions (1:150) to which spin-labelled stearic acids were added are depicted in Fig. 10. EPR, ^{1}H- and ^{13}C-NMR data showed that the labels were incorporated into lysolecithin micelles and lysolecithin/basic protein complexes. Addition of small amounts of spin-labels to PLPC/MBP complexes did not change the chemical shifts of assigned resonances, indicating that, at least at the low concentrations used, the stearic acid labels did not perturb the conformational characteristics of the lysolipid/protein aggregates. The presence of the 5- and 16-doxylstearate spin-labels affected differently the resonances of various methyl groups. The methyl resonance of the *N*-Ac-CH_3 peak was minimally affected by either label, the linewidths of the Met-176-CH_3, Met-21-CH_3, Thr-66-CH_3 and Thr-67-CH_3 increased slightly with both labels; the Val-87-CH_3, Val-88-CH_3 and methyl resonances were broadened by the 16-doxyl spin-label. The presence of the doxylstearate labels also produced small selective effects on aromatic resonances. No increase in the linewidth was measured for the observable His-ε_1-CH resonances, Tyr-69 or Tyr-128-ε, δ-CH_2, but broadening occurred in the spectral lines corresponding to Trp-117 and those arising from phenylalanine residues in the region 7.0–7.5 ppm.

Protein/lysolipid mixtures at a molar ratio 1:200 were lyophilized from H_2O and resuspended in ^2H_2O for NMR measurements. All exchangeable protons were undetectable after 30 min, indicating that the binding of MBP to PLPC micelles did not protect any peptide or amide hydrogens from rapid exchange with the surrounding water.

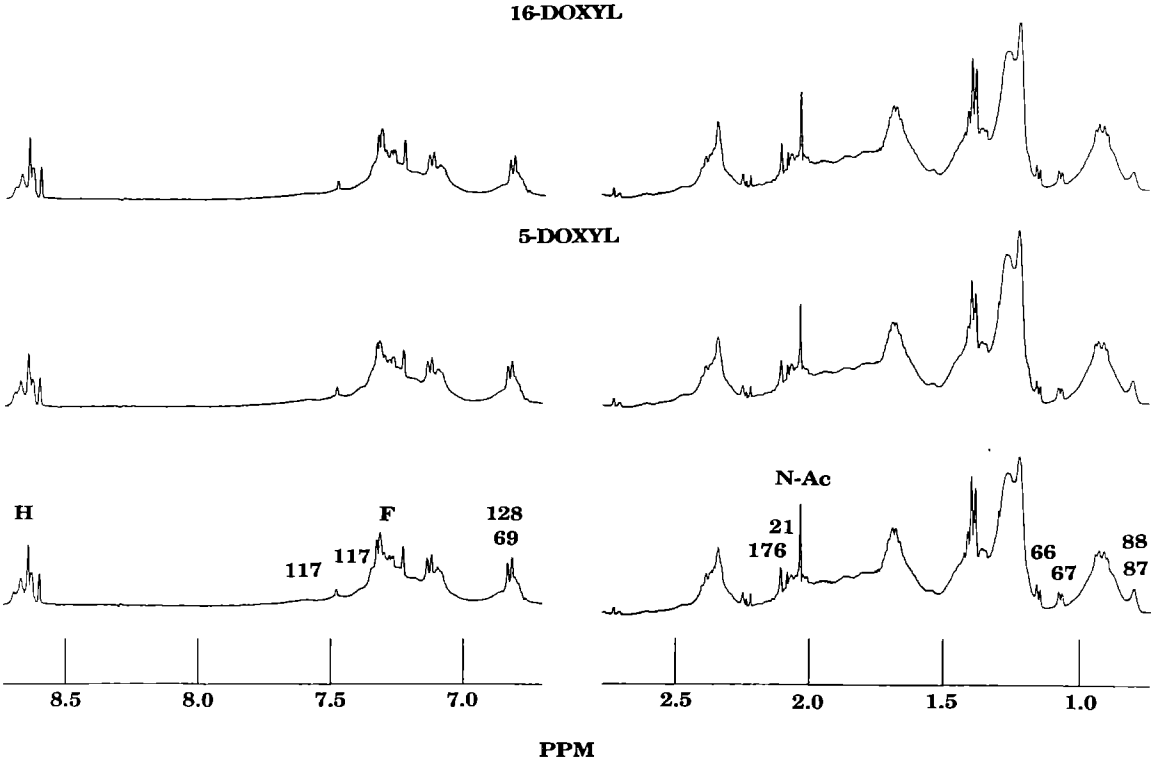

Fig. 10. Two regions of the ^{1}H-NMR spectra of myelin basic protein showing the effects of incorporation os spin-labelled fatty acids into PLPC/MBP complexes in aqueous solutions. The ratio [PCLP]:[MBP] was 120:1; spectra were acquired at pH 3 and 298 K. *Bottom:* no spin-label; *middle:* 5-doxylstearate, *top:* 16-doxylstearate. The specific assignments of some resonances are indicated on the figure by residue number. Histidyl and phenylalanyl resonances are indicated by H and F, respectively; N-Ac is the resonance arising from the N-terminus acetyl group

Discussion

An investigation of the interactions of myelin basic protein with micelles of lysolecithins of different acyl chain lengths demonstrated that the changes induced in the conformation of the protein molecules depended on the length of the acyl chain, and were maximal for the palmitoyl lysolipid (Mendz et al. 1992). The present study provides direct evidence that PLPC/MBP complexes consist of polypeptide chains bound to lysolipid molecules that retain a micellar organization.

Ultracentrifugation data indicated that the basic protein forms a complex of well-defined size and stoichiometry with palmitoyllysolecithin micelles. The aggregates consisted of one protein molecule bound to one lysolecithin micelle with a size of approximately 141 lysolipid molecules. Within experimental error this value is equal to the values 145 and 133 found for MBP complexes with dodecylphosphocholine (DPC) (Mendz et al. 1988) and dodecylphosphocholine/palmitoyllysophosphatidic acid (DPC/PLPA) (Mendz et al. 1991), respectively. Since in DPC and DPC/PLPA suspensions the composition of the complexes is one protein molecule bound to two detergent micelles, it appears that a basic requirement of these complexes is a stoichiometry of approximately 150:1 lysolipid:basic protein molecules.

The small-angle X-ray scattering results indicated that the radius of gyration of the lysolipid micelles was 3.5 ± 0.5 nm, a value slightly larger than 3.4 nm determined for PLPC micelles (Haberland and Reynolds 1975) and similar to 3.5–4.0 nm obtained for LPC micelles (Perrin and Saunders 1964). The radius of gyration of the protein/lysolipid complexes was 3.8 ± 0.4, indicating that the binding of the protein resulted only in small changes in the PLPC micelle size. EPR data indicated that both spin-labels were incorporated into lysolecithin micelles and lysolecithin/basic protein complexes. NMR (Figs. 6 and 7) and EPR results (Table 2) established that the labels caused no appreciable perturbation of the organization of the lysolipid/protein aggregates. The data on micelles and aggregates indicated that the presence of the protein did not induce a significant change in the size of the micelles, and that the environment and location of the nitroxide moieties of the micelle-bound spin-labels were affected little by the presence of myelin basic protein.

Addition of the spin-labels induced selective broadening of carbon resonances in the carbon spectra of PLPC and PLPC/MBP suspensions. The presence of 5-doxylstearate increased the linewidth of the peaks arising from carbon nuclei 4–15 (Fig. 6), with maximal effect on the proton nuclei resonances at positions 14 and 15 (Fig. 7). The 16-doxylstearate spin-label increased the linewidth of carbon nculei at positions 1, 2 and 3, and had maximal effect on the proton resonance at position 1 (Fig. 7). These results indicated that the locations of the labels in the lysolipid micelles were not altered significantly by the presence of the protein, and suggested that the 5-doxylstearate was near the interface and the 16-doxylstearate deep

in the hydrocarbon core. The selective broadening of only a few ^{1}H-NMR resonances of the protein spectrum (Figs. 9 and 10) by the presence of the spin-labels, suggested that the polypeptide backbone is mostly outside the micellar surface, with only specific regions of the protein molecule penetrating into the micelles at various depths. The larger paramagnetic contributions observed in suspensions containing MBP (Figs. 6 and 7) could be explained by "crowding" of the interior of micelles by protein segments, which would result in diminished rates of motion of spin-labels within the micelles. The EPR data on ^{14}N hyperfine splitting and correlation times of the nitroxide groups in micelles and complexes (Table 2) supported this interpretation.

Addition of MBP did not produce significant changes in the ^{13}C longitudinal relaxation times of the carbon nuclei of PLPC (Table 3), indicating that the perturbations occurring by the binding of the protein to micelles did not affect the rapid components of the lysolecithin chain motions with correlation times in the order of 10^{-9}–10^{-8} s. The presence of the protein induced decreases in the ^{13}C transverse relaxation times of the carbon nuclei of the acyl chain of PLPC up to position C_{14} (Table 3), which are visualized by broadening of some the resonances in the spectrum of lysolecithin (Fig. 6). The results suggested that the lysolipid-protein interactions introduce new slow motions of the PLPC molecules which are capable of modulating the dipolar interactions at certain regions of the acyl chain sufficiently to influence T_2 but which are too slow to affect T_1. These effects were similar to those measured in DPC/MBP complexes (Mendz et al. 1988), but were not observed in DPC/PLPA/MBP aggregates (Mendz et al. 1991).

The association of the basic protein with lysolecithin micelles increased the longitudinal and transverse relaxation times of the phosphorus nuclei in the headgroup (Table 3). The EPR and X-ray scattering data on the size of the PLPC micelles and PLPC/MBP aggregates suggested that these changes in the relaxation times cannot be ascribed to variations in the size of the micelles. The perturbations in the motion of this group are quite different from those observed in DPC/MBP complexes and DPC/PLPA/MBP aggregates where the presence of the basic protein *decreases* the relaxation times of the phosphorous nuclei. If most of the protein were located at the micellar surface, rearrangements of the lysolecithin headgroups must take place to make room for the polypeptide. The presence of the protein would restrict the motions of the headgroups *decreasing* the ^{31}P relaxation times, as was observed in DPC/MBP complexes (Mendz et al. 1988) and DPC/PLPA/MBP aggregates (Mendz et al. 1991). Similarly, the effects of chemical exchange of these nuclei between different sites would be, in general, a broadening of the spectral lines, resulting in *decreases* in the relaxation times. In PLPC micelles the relaxation times of the glycerol backbone and choline headgroup ^{13}C nuclei did not change after addition of MBP (Table 3), suggesting that the increases in ^{31}P relaxation times arose from some specific property of these nuclei. The absence of significant changes in the relaxation rates of the ^{13}C nuclei could be attributed to the relaxation mechanisms of these atoms which are dominated by dipolar interactions with bonded protons, and may not be sensitive enough to slight reorganizations of the surface of the micelles which could bring about small changes in overall motion, orientation and/or environment of the headgroups.

Restrictions to the motions of the phosphoryl groups may arise from steric hindrance by neighbouring headgroups and/or the formation of intermolecular hydrogen bonds or salt bridges between ionizable moieties of the lysolipid at the micellar interface. Since phosphorylcholine groups only interact ionically by charge neutralization (Boggs and Moscarello 1978), it is possible that the faster motions of phosphorus nuclei result from a lessening of steric hindrances between headgroups. A decrease in headgroup restraints may occur by separation of lysolipid molecules wrougth by intercalation of segments of the protein into the micelle core, with effects analogous to those of incorporation of cholesterol into phospholipid micelles (Tanford 1973). On the other hand, the presence of the protein at the micelle surface will limit the extra space created at the interface, making it insufficient to induce a significant change in the motion of the carbon nuclei of the lysolipid headgroup owing to the much larger volume swept by the choline moiety. The observations that incorporation of chloroform into PLPC micelles or PLPC/MBP complexes did not change the relaxation times of the ^{13}C nuclei of the glycerol backbone and phosphoryl choline or the relaxation times of the headgroup ^{31}P nuclei did not support the explanation that the faster motions of phosphorus nuclei observed in the presence of MBP in the complexes arose from separation of lipid molecules by the intercalation segments of the basic protein into the core of the micelles.

The faster motions measured for the phosphoryl group may relate to the change in the molecular environment of the PLPC headgroups caused by the presence of MBP. A difference between the structures of DPC and PLPC is the existence of a glycerol backbone in the lysophosphatidylcholine. The extra length provided by the PLPC backbone may be sufficient to accommodate the protein without requiring major changes in the motions of the headgroups. In the phosphoryl moiety the phosphorus atom is bonded to oxygen atoms which would not contribute to the relaxation rates of ^{31}P nuclei. As a consequence, although relaxation through dipole-dipole interactions with neighbouring protons will be present, relaxation via chemical shift anisotropy (CSA) becomes important (Vogel 1989). This relaxation is caused by the modulation of an anisotropic nuclear screening tensor such as that present in the phosphoryl group in the phosphatidylcholine moiety (Silver 1985). In hydrated dipalmitoylphosphatidylcholine bilayers the motion of the headgroup can be modelled with a tensor with cylindrical symmetry about an axis normal to the bilayer (Vogel 1989), and the relaxation rates are proportional to the square of the shielding anisotropy, that is, the difference between the parallel and perpendicular components of the axially symmetric tensor (Harris 1983). Although substantial averaging of this tensor will occur in micelle suspensions, it is reasonable to assume that the CSA relaxation rates would remain approximately proportional to the shielding anisotropy. Environmental effects

have been reported to have small effects on the shielding tensor of ^{31}P nuclei; in particular the spectral positions of phosphate esters are sensitive to solvent effects (Lerner and Kearns 1980). The presence of the protein at the interface could interfere with the environment of the headgroups decreasing the shielding anisotropy, and thus giving rise to faster relaxation times. It is also possible that the presence of the protein may induce a change in the orientation of the phosphoryl group, thus changing the motionally averaged CSA tensor and resulting in faster relaxation rates.

The CD and NMR results demonstrated that conformational changes occur in the basic protein and peptides as a result of their interactions with the lysolecithin (Fig. 1), that there are discrete binding sites in the MBP molecule for PLPC, and that the polypeptides bound preferentially to lysolipid micelles rather than isolated molecules (Fig. 2). The results are in agreement with those obtained for dodecylphosphocholine (Mendz et al. 1984), dodecylphosphocholine/palmitoyllysophosphatidic acid (Mendz et al. 1990), and for myristoyl- and palmitoyllysophosphatidylcholine (Gow et al. 1990). Important features of the ^{1}H-NMR protein spectra of PLPC/MBP complexes were the relatively narrow linewidths of resonances (Fig. 8); the generally small changes in chemical shifts and the increase in linewidths of specific residues compared to the spectra in aqueous solutions (Fig. 9); the ability of the spin-labels to broaden resonances from residues at very different positions in the MBP amino acid sequence (Fig. 10); and the lack of protection afforded by the lysolipid against exchange of the protein amide protons with $^{2}H_2O$. These observations supported the conclusions that most of the interactions of MBP with PLPC are confined to the interfacial region of the micelles and that the parts of the polypeptide chain that penetrate the micelle move rapidly between lipid and aqueous environments.

Specific protein residues involved in the interactions with the lysolipid can be designated from the NMR data on chemical shifts, linewidths and spin-label linebroadening. The interactions with PLPC are not restricted to any one region of the MBP polypeptide although appreciable effects are more frequent in the N-terminal half of the chain. Sites of possible amphipathic helices in myelin basic protein have been identified in the sequences comprising residues 13–28, 35–45, 58–73, 87–97, 108–119 and 140–159 (Mendz et al. 1990). Considering that the actual number of protein residues in α-helical conformations in PLPC/MBP aggregates (Table 1) is smaller than the number of residues capable of forming amphipathic helices, there are several possible locations for the helices. A broad outline of helical segments was suggested on the basis of the interactions of the protein with lysophosphatidylcholines with different acyl chain length (Mendz et al. 1992). In the present study the CD data on peptide fragments was employed to delineate the regions that become α-helical in the whole protein, allowing a more definitive elucidation of the location of MBP segments which adopt helical conformations in the association with PLPC.

In the region 13–28 of MBP, the broadening of the resonances arising from Tyr-14-δ-CH_2 and Met-21-ε-CH_3 (Fig. 9) and the negligible effects on the His-26-ε_1-CH peak (Mendz et al. 1992) suggested that the sequence Tyr-Leu-Ala-Thr-Ala-Ser-Thr-Met-Asp-His comprising residues 14–23 may be involved in the interactions with PLPC. NMR studies of the associations of MBP with other lipids have identified Met-21 as a locus for lipid interactions (Deber et al. 1978; Littlemore and Ledeen 1979). An analysis of the protein sequence by helical wheels (Schiffer and Edmundson 1967; Randall and Zand 1985) and space-filling models which yielded an amphipathic helix with its boundaries at Tyr-14 and His-23, and the sequences Ala-Thr and Thr-Met on the hydrophobic side of the helical wheel, supported the conslusion that part of the segment 13–28 may adopt a helical conformation in the bound protein. The strong change in the chemical shift of His-10-ε_1-CH in the protein bound to lysolecithin could be explained by the loss of a local conformation existing in the protein in aqueous solution which in a turn of the backbone brings Tyr-14 close to His-10, producing ring current effects that induce a shift in the histidyl resonance (Mendz et al. 1985a, 1986). Binding to lysolecithin could abolish the interaction between these two residues.

The regions 35–45 and 58–73 have been identified as possible sites of amphipathic helices (Mendz et al. 1990). There are no markers for the segment 35–45 which would provide direct information on it. In the segment 58–73 the resonances from His-62, -63 and -68, Thr-66 and -67 and Tyr-69 serve as markers. The small changes observed on these resonances in the formation of PLPC/MBP complexes (Figs. 8, 9 and 10) could be ascribed to generalized effects resulting from the presence of lysolecithin in the suspensions. Since helical wheel analysis of this region places Tyr-66 and Tyr-67 on the hydrophobic side of the wheel, formation of an amphipathic helix would have involved the threonine residues in specific interactions with the lysolipid micelles, resulting in strong effects on the threonyl resonances. Thus, it does not appear that an α-helix is formed segment 58–73.

Cleavage of MBP at residue Phe-90, in the most hydrophobic region of the protein, resulted in fragments, 1–90 and 91–179, of similar size. In PLPC suspensions the total number of residues in helical conformations was 34 for the intact protein and 24 for the sequential peptides 1–90 and 91–179 (Table 1). The smaller number of residues in helices in the peptides may be accounted for by the 10 residues that could form an amphipathic helix in segment 87–97 of the whole protein, but not in the peptides where the segment has been divided by the cleavage. In contrast, the number of residues in helical conformations in lysolecithin suspensions of peptides 1–98 and 99–179, resulting from cleavage of MBP at Arg-98, is approximately the same as for the intact protein. These data underlined the importance of the integrity of the segment 91–98 for the formation of a helix in PLPC suspensions, as was the case for the detergent dodecylphosphocholine (Mendz et al. 1984) and dodecylphosphocholine/palmitoylphosphatidic acid mixtures (Mendz et al. 1990). Thus in agreement with the previous analysis (Mendz et al. 1992), it is reasonable to conclude that residues 87–97 are included in an α-helical region. This conclusion was supported by the strong broadening of the methyl resonances of Val-87 and Val-88 observed when the 16-doxylstearate spin-label was incor-

porated into the lysolipid micelles (Fig. 10), and agrees with studies of the conformation of the protein in nonpolar milieux as models for lipid environment (Stone et al. 1985; Whitaker et al. 1990).

Trp-117 is a good marker for the segment 108–119 capable of forming an amphipathic helix (Mendz et al. 1990). The linewidth increase of the Trp-ε_3-CH and Trp-ζ_2-CH resonances in the presence of PLPC (Figs. 8 and 9) and the further broadening by 16-doxylstearate indicated that the aromatic moiety interacted with the losylipid. A helical wheel of this segment places the tryptophan residue on the hydrophilic side of the wheel (Mendz et al. 1990). The next two residues are a glycine and an alanine, the former constitutes the boundary of the amphipathic helix, followed by the alanyl located on the hydrophobic side of the wheel. A small distortion of the end of the helix would allow the bulky tryptophanyl side chain to penetrate the lysolipid micelle. The broadening of the tryptophanyl resonances was thus consistent with the formation of an α-helix in this region.

The last amphipathic helix has been identified in the segment 140–159 for which no markers are available; but the resonances of Tyr-128, Tyr-135 and His-139 in the region preceding the segment were affected by the formation of PLPC/MBP complexes (Figs. 8 and 9). Monoclonal antibodies have been raised against an epitope located in the region 130–140 (Hruby et al. 1985), and there is evidence of a defined secondary structure in this region (Mendz et al. 1985b). CD investigations showed that the segment 130–140 formed an α-helix at 30% trifluoroethanol concentration (Martenson et al. 1985), in a transition from an epitopic region in aqueous solution to α-helix in a nonpolar environment, similar to that observed for the segment 87–97 (Whitaker et al. 1990). It is then possible to conclude that at least part of the segment 130–140 adopts α-helical conformation in the PLPC/MBP aggregates.

In summary, the results suggested that the twenty residues in α-helices in peptide 1–98 bound to lysophosphatidylcholine micelles (Table 1) are likely be located in segments 13–28, and 87–97, although not every residue in these regions would form part of a helix. The twelve residues in α-helical conformations in peptide 99–179 bound to lysolecithin (Table 1) are probably located in segments 108–119 and 130–140.

MBP binds strongly to anionic surfactant micelles in aqueous suspensions (Mendz et al. 1990, 1991; Burns et al. 1981; Burns and Campagnoni 1983) and to reverse micelles in organic solvents (Nicot et al. 1985; Chatenay et al. 1985). The basic protein also binds to zwitterionic surfactant micelles (Mendz et al. 1984, 1988; Smith 1982; Gow et al. 1990) and reverse micelles (Nicot et al. 1985). In the association with both types of detergents there are significant differences in the protein-lipd interactions, the physical properties of the complexes and the conformations adopted by the protein. In the specific case of lysophospholipids there were similarities between the interactions of MBP with DPC (Mendz et al. 1984, 1988), DPC/PLPA (Mendz et al. 1990, 1991) and PLPC, but there were also important differences. At variance with DPC/MBP and DPC/PLPA/MBP aggregates, only one lipid micelle was present in PLPC/MBP complexes and no supramolecular structures were observed in electron micrographs of these complexes, suggesting that the presence of a glycerol backbone in palmitoyllysophosphatidylcholine makes an important difference to the association with myelin basic protein.

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