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Effects of acyl chain length on the conformation of myelin basic protein bound to lysolipid micelles

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Abstract

The interactions of myelin basic protein with micelles of lysophosphatidylcholine detergents of different acyl chain lengths were investigated by circular dichroism (CD), small-angle X-ray scattering, Fourier transform infrared spectroscopy (FT-IR), and ^{1}H, ^{13}C and ^{31}P nuclear magnetic resonance spectroscopy (NMR). Circular dichroic, FT-IR, and ^{1}H NMR measurements indicated that the conformational changes induced in the protein molecules by association with micelles depended on the acyl chain length of the detergents. Size is one of the physical properties of micelles which is a function of the length of the acyl chains. The radii of gyration of detergent micelles in complexes with the protein measured by small-angle X-ray scattering indicated that the average size of the micelles was a quadratic function of the acyl chain length. The dependence of the protein conformational changes on micelle size was used to ascertain the order in which different protein segments associate with the detergents. Several procedures were employed to change the fluidity of micelles formed with detergents of given acyl chain lengths. The conformational changes observed on the MBP molecule by varying the micelle properties without changing the length of the chain, suggested that the changes depended on the size and fluidity of the micelles.

Keywords: Protein–lipid interactions; Myelin basic protein; Lysophosphatidylcholine; Spectroscopic techniques (NMR, CD, FT-IR, SAXS)

1. Introduction

The conformations adopted by proteins in association with lipids depend on the location of the protein molecule relative to the lipid matrix, the intermolecular interactions between lipid molecules, and the mechanisms binding proteins to lipid bilayers. These are interrelated factors which have been extensively investigated for integral membrane proteins [1], but are less well known for extrinsic membrane proteins such as myelin basic protein (MBP).

Owing to the important influence of the environment on the spatial organization of MBP, its conformations in aqueous solutions are generally different from those in organic solvents or in association with lipids or detergents. Thus, the structure of the basic protein in myelin, where it is at the interface of a solid phase and an aqueous phase, is likely to be different from the con-

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formations it adopts in a physiological liquid, where MBP can act as an antigent interacting with receptors in lymphocytes.

Basic information required to understand the molecular architecture and functions of the myelin membrane have been provided by studies of the interactions of MBP with lipids found in the myelin membrane such as phospholipids, sphingolipids and glycolipids, and with reconstituted mixtures of these lipids [2–5]. The influence of the lipid head group on the binding of the basic protein has been examined in model systems with neutral and charged phospholipids, and glycolipids [6,7]. Investigation of the dependence of the association of MBP with the length of the acyl chains of phosphatidylglycerol bilayers showed that the lipid could freeze into a stable state more readily if a smaller proportion of the total bilayer thickness was occupied by hydrophobic segments of the protein [8].

Although the associations of proteins with detergent micelles only model protein–lipid bilayer interactions in a limited fashion, in the case of myelin basic protein they have provided a wealth of information about the nature of the conformational changes induced on the protein, its location relative to the detergent–solvent interface, the nature of the binding, and the role of electrostatic and hydrophobic interactions [9–12]. Investigations of the effects of the acyl chain length of lysophosphatidylcholines on the interactions with MBP showed that the concentration at which the binding commences is markedly influenced by the chemical structure of the detergents, and that the hydrophobic nature of the association was unaffected by the length of the acyl chain [13].

The present work investigated the effects on the conformations of the basic protein of its association with lysophosphatidylcholine micelles of detergents with acyl chains from 6 to 18 carbon atoms, and with detergent micelles whose properties were changed by several methods.

2. Materials and methods

Caproyllysophosphatidylcholine (LPCC), capryllysophosphatidylcholine (LPCD), lauroyllysophosphatidylcholine (LPCL), myristoyllysophosphatidylcholine (LPCM), palmitoyllysophosphatidylcholine (LPCP), stearoyllysophosphatidylcholine (LPCS) and oleoyllysophosphatidylcholine (LPCO) were obtained from Serdary Research Laboratories Inc. (London, Ontario). Lysophosphatidylcholine from bovine brain (LPC), containing primarily palmitoyl, stearoyl, and oleoyl moieties was purchased from Sigma (St. Louis, MO). Lysolipids were used without any further purification. Rabbit and bovine MBP were prepared according to the method of Law et al. [14]. Porcine MBP was kindly provided by Dr Max Marsh of Eli Lilly & Co. 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. [9]. This convention was adopted to maximize the homologies in the primary structure of proteins derived from different species.

2.1 Circular dichroism (CD)

Circular dichroic spectra were measured at 298 K with a JEOL-500C spectropolarimeter at various detergent/protein ratios. Spectral range was 195–275 nm, and 4–16 spectra were accumulated over 8 K 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 concentration was 8×10^{-6} to 1×10^{-4} *M*; detergent concentrations ranged from 3.3×10^{-4} to 18×10^{-3} *M*. Measurements were carried out in 0.05 *M* phosphate buffer, pH 7.0. The CD spectra of the detergents alone were negligible over the spectral range recorded. In the range of concentrations employed, the mixed detergent/protein suspensions were optically clear. Sonication in a bath after addition of chloroform or n-hexanol to the samples initially produced turbidity in the suspensions, but the samples became clear again after the organic solvents were incorporated into the detergent micelles. The α-helix content of the protein was calculated 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 [15]. Absolute values of α-helicity are uncertain to about $\pm 4\%$, but comparative values are reproducible to $\pm 0.5\%$. The absolute values of the calculated β-sheet content of the spectra are uncertain to about $\pm 15\%$, and the errors in the values determined for nonordered structures are considered larger than $\pm 15\%$ [15].

2.2 Fourier transform infrared spectroscopy (FT-IR)

Protein/detergent suspensions at molar ratios 1:150 ($\times 10^{-3}$ M) were prepared in 0.05 M ^2H_2O phosphate buffer, pH 7.0. To minimize the water content, proton–deuterium exchange was achieved by lyophilizing and dissolving the suspensions twice in 99.5% ^2H_2O (Australian Institute for Nuclear Science and Engineering, Lucas Heights, NSW). Samples were placed in a 12 μm CaF_2 cell and examined in a Digilab FTS-80 spectrometer with a Glowbar source and a HgCdTe detector. In order to eliminate spectral contributions of atmospheric water vapor, the instrument was continuously purged with pure dry nitrogen gas. Spectra were acquired at 298 K in the absorbance mode against air background. To ensure adequate signal-to-noise ratio 1024 interferograms were accumulated. These were apodized with a triangular function and Fourier transformed to give a resolution of 2 cm^{-1}. The use of spectral deconvolution and derivative spectroscopy to resolve overlapping bands and obtain their frequencies, and of curve fitting analysis to determine the fractional areas of each component, make possible the identification of the specific components of protein secondary structure, but without solving the problem of determining the uniqueness of the values calculated for the contributions of each component band to the overall spectral envelope. The computational procedures for spectral deconvolution of this region of the spectrum of proteins ordinarily employ six to nine component bands whose spectral positions are characteristic of β-structures, turns, α-helix and non-ordered segments of polypeptide chains [16]. However, the spectra of α-casein, which appears to have very little secondary structure in aqueous solutions, and of myoglobin, a highly helical protein, were deconvolved employing only three bands; and the spectra of haemoglobin and cytochrome c were deconvolved with five bands [16]. The spectra of myelin basic protein in aqueous solutions and in dimyristoylphosphatidyl glycerol suspensions were analyzed using five component bands [17]. Several attempts at deconvolution and curve fitting of the MBP spectra in detergent suspensions with five component bands did not yield coherent solutions. It was possible, however, to obtain consistent analyses employing only four bands: two for β-structures, one for non-ordered segments and one for turns and α-helices.

2.3 Nuclear magnetic resonance (NMR)

Protein and protein/detergent suspensions were lyophilized twice from 99.5% ^2H_2O (Australian Institute for Nuclear Science and Engineering, Lucas Heights, NSW) and dissolved in 99.96% ^2H_2O (Merck, Sharp & Dohme, Montreal) at protein concentrations 0.5–1.0 mM in 5-mm OD precision NMR tubes (Wilmad, Buena, NJ). The pH was adjusted with ^2HCl or NaO^2H and measured with a thin Activon BJ331 combination glass electrode, values being reported as meter readings. Increasing amounts of detergent were added to the protein solutions to obtain a series of detergent/protein suspensions at molar ratios from 0 to 150. The ^1H NMR spectra at 400 MHz and ^{13}C NMR spectra at 100.5 MHz, were measured on a Bruker WM-400 or a Varian XL-400 spectrometer, operating in the pulsed Fourier transform mode with quadrature detection. ^1H NMR spectra of porcine MBP were measured at temperatures between 278 and 338 K over 16 K data points; acquiring 1000–2000 transients with radiofrequency pulses of 90° (7–15 μs), spectral width of 4000 Hz, and a repetition time of 2.1 s. To avoid dynamic range problems in measuring protein spectra against a background of lysolipid resonances at high lysophosphatidylcholine/protein molar ratios, the detergent resonances corresponding to the choline methyl protons and the methyl and methylene protons of the acyl chains were presaturated for 0.03 s. Spectra were trans-

formed by employing either no window functions or Gaussian multiplication with line broadenings of −1 to −4 Hz and a Gaussian broadening factor of 0.19. Chemical shifts were measured relative to internal DSS (sodium 4,4-dimethylsilapentane-1-sulfonate) by the method previously described [18]. Relaxation measurements of the ^{13}C resonances of detergent suspensions with or without MBP, were performed at 300 K by averaging 256 transients over 16 K data points per spectrum, with spectral width of 6920 Hz, radiofrequency pulses of 90° (23 μs), and a repetition time of 20 s. Free induction decays were transformed employing a linebroadening of 3 Hz. Longitudinal relaxation times (T_1) were measured by the inversion-recovery pulse sequence [19] with delay times of 0.001-20 s, and arrays of 18 spectra were collected with interleaved acquisitions for each experiment. T_1 values were calculated by non-linear regression analysis [20]. The assignments of ^{13}C lysolipid resonances are those of Levine et al. [21]. The ^{31}P NMR spectra were measured at 121.6 MHz in a Bruker CXP-300 spectrometer, operating in the pulsed Fourier transform mode with quadrature detection. Spectra of protein/lysolipid mixtures were acquired at temperatures between 278 and 318 K, by averaging 80 transients over 16 K data points. A radiofrequency pulse of 90° (19 μs) was used, with a spectral width of 1000 Hz and a repetition time of 40 s. Free induction decays were transformed with a linebroadening of 1 Hz.

2.4 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 equilibrate for over 24 h. Measurements were carried out using the high resolution SAXS camera at the Research School of Chemistry, The Australian National University [22]. The instrument employs 0.15 nm X-rays, has a 1 m focal length, and wavelength selection and focussing 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 focussing 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 [23]. 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 [24]. Intensities were measured as functions of the momentum transfer Q, with

$$Q = (4\pi \sin \theta)/\lambda \quad (1)$$

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

3. Results

3.1 Circular dichroism (CD) of protein / detergent mixtures

CD spectra of bovine basic protein/detergent suspensions at molar ratios 1:150 measured in phosphate buffer are shown in Fig. 1; increasing the amount of lysolipid in the suspensions beyond this ratio did not result in any further spectral changes. The results of the analyses of the protein spectra in terms of secondary structures are given in Table 1. Varying the concentration of MBP between 8×10^{-5} and 1×10^{-4} *M* did not yield changes in the spectra of the protein/lysolipid suspensions. The measurements indicated that conformational changes induced in the protein molecules by association with detergent micelles depended on the acyl chain length of the lysolipids.

Non-polar compounds are commonly solubilized into micelles by simple partitioning between the liquid-like apolar micellar interior and the aqueous environment in the intermicellar solution [25]. Chloroform was incorporated into detergent/protein complexes by sonication after addition to MBP/lysophosphatidylcholine–water

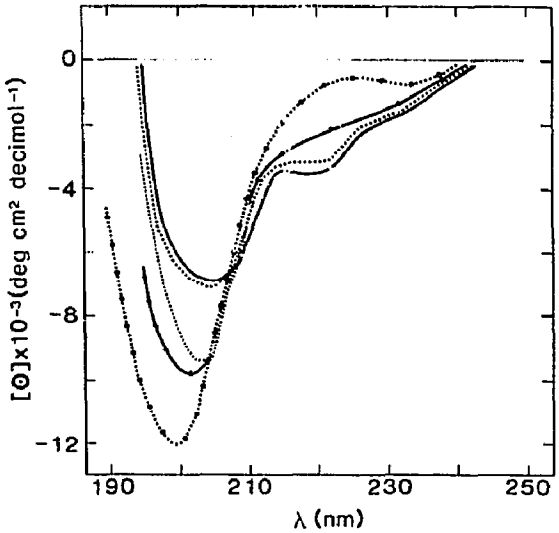

Fig. 1. Circular dichroic spectra of 1:150 MBP/detergent mixtures at 298 K in 0.05 *M* phosphate buffer, pH 7. MBP in aqueous solution (○·····○); MBP/LPCC (△———△); MBP/LPCD (— — —); MBP/LPC (·····); MBP/LPCP (———).

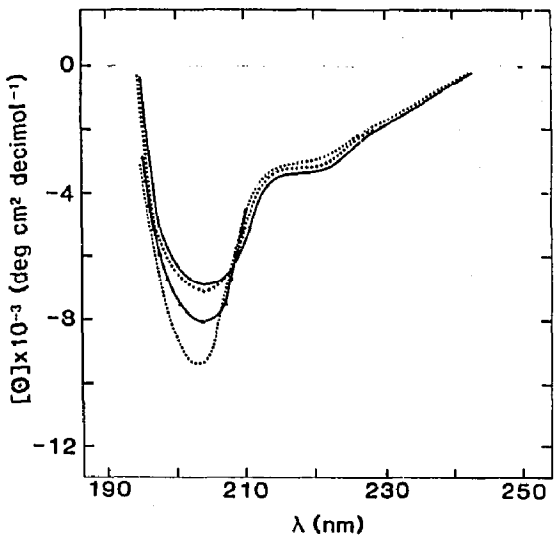

Fig. 2. Circular dichroic spectra of 1:150 MBP/LPCD at 298 K in 0.05 *M* phosphate buffer, pH 7, with $CHCl_3$ added to the suspensions. The chloroform-to-detergent molar ratios were: 0:1 (–·—·—) 5:1 (△———△); 25:1 (·····); and 50:1 (———). At wavelengths above 215 nm the 0:1 and 5:1 spectra overlap.

suspensions. Addition of chloroform and mixing made the suspensions cloudy initially, but they became clear again after sonication, suggesting that the chloroform was incorporated into the lysolipid micelles. The presence of $CHCl_3$ in the complexes induced changes in the CD spectra of most detergent/protein mixtures; Fig. 2 shows the spectral changes observed for MBP/LPCD dispersions. Titrations of basic protein/lysolipid suspensions showed that in most instances the α-helicity of the polypeptide chain increased with the amount of chloroform incorporated into the complexes (Fig. 3). The increases in α-helical content of the basic protein molecules by addition of chloroform depended on the length of the

Table 1

Molar percentage of β-structure, random and α-helix and number of α-helical residues of bovine MBP in several detergents at 298 K and pH 7 in 0.05 *M* phosphate buffer

Detergent	β-Structure	Random	α-Helix	α-Helical residues
None	6	89	5	8
LPCC	10	84	6	10
LPCD	11	79	10	17
LPCL	12	75	13	22
LPCM	16	68	16	28
LPCP	17	63	20	34
LPCS	13	71	15	25
LPCO	15	71	13	22
LPC	12	77	11	19

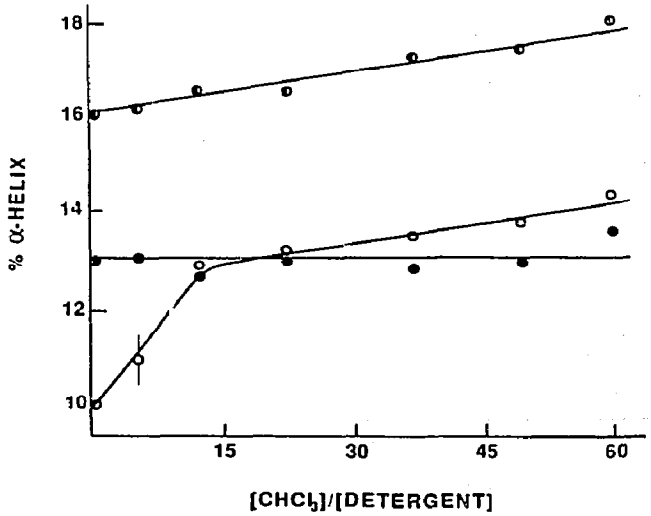

Fig. 3. Calculated percent α-helicities of myelin basic protein as a function of chloroform concentration in 1:150 MBP/LPCD (○), MBP/LPCM (◐), and MBP/LPCS (●) mixtures at 298 K in 0.05 *M* phosphate buffer, pH 7.

acyl chain of the lysolipid in the suspension. The changes in α-helix were smaller for the detergents with the longer chains; and incorporation of $CHCl_3$ into MBP/LPCS complexes did not affect the CD spectra of the suspensions (Fig. 3).

For partly polar compounds such as alcohols there is no simple description of the process of solubilization into detergent micelles, but polar groups are expected to be located near the surface, and for long-chain alcohols a location close to the micelle surface of the polar group, and an orientation of the acyl chain towards the micelle centre could be anticipated [25]. Titrations of MBP–lysophosphatidylcholine–water suspensions with methanol or n-hexanol induced changes in the CD spectra of the suspensions. The incorporation of either alcohol into protein/detergent complexes decreased the α-helical content of the MBP molecules in most cases. The changes in secondary structure measured for n-hexanol titrations were larger than for methanol titrations at comparable alcohol-to-detergent molar ratios (Fig. 4). The presence of alcohol in the complexes affected more the conformations of the basic protein in suspensions of lysophosphatidylcholines with longer acyl chains than in suspensions of lysolipids with shorter acyl chains (Fig. 4).

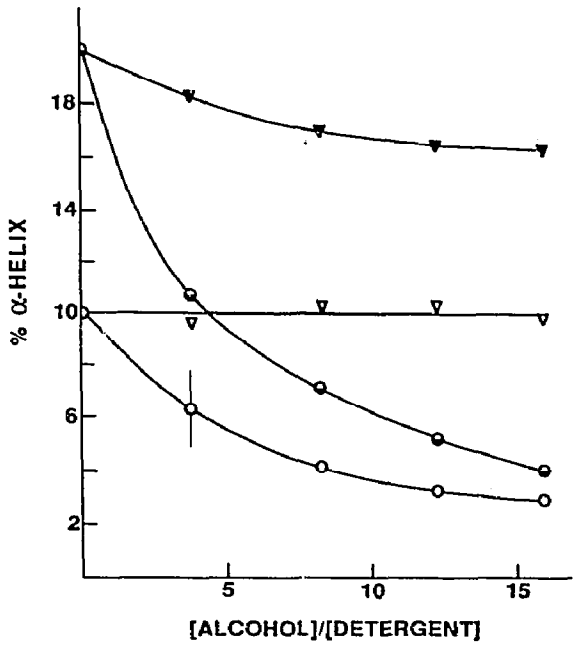

Fig. 4. Calculated percent α-helicities of myelin basic protein as a function of alcohol concentration in 1:150 MBP/LPCD (open symbols), MBP/LPCP (full symbols) mixtures at 298 K in 0.05 *M* phosphate buffer, pH 7. The (○) symbols refer to titrations with n-hexanol and the (▽) symbols to titrations with methanol.

3.2 FT-IR of protein / detergent suspensions

The amide I region of the infrared spectra of myelin basic protein in suspensions of LPCC and LPCS are shown in Fig. 5. This band has become a useful source of information about the conformations adopted by proteins in solution because of its sensitivity to the different forms of backbone secondary structure. However, the analysis into components and quantification of the amide I band of polypeptides is not simple because this region of the infrared spectrum presents a broad and featureless contour, and the constituent vibrational bands are also broad and overlap. Overlapping infrared bands were resolved employing spectral deconvolution and derivative spectroscopy. Curve fitting was performed by using the procedure of Fraser and Suzuki [26]; the best results were obtained by fitting the amide I region of the spectra to four component bands centred at approximately 1630, 1641, 1651 (or 1659), and 1674 cm^{-1}, corresponding to β-structures, non-ordered structures (random), α-helices and turns, and β-structures, respectively [16]. Attempts at fitting the spectra with separate bands for the contributions arising from α-helices and turns were unsuccessful. Two extra bands of undefined origin at approximately 1584 and 1610 cm^{-1}, outside the amide I region, appeared consistently in the fitted spectra. The fits of component bands to the spectra of MBP/LPCC and MPB/LPCS suspensions are shown in Fig. 5, and the results of the analyses for all the protein/detergent suspensions examined are given in Table 2. The total β-structure content of the MBP molecule measured for the 1630 and 1674 cm^{-1} bands remained constant at approximately 41% for all the protein/detergent complexes. The content of α-helices and turns measured for the 1651 (or 1659) cm^{-1} band increased from 26%

for protein/LPCC mixtures to 37% for protein/LPCP suspensions.

3.3 ^1H NMR spectra of protein/detergent complexes

At neutral pH myelin basic protein aggregates even at low concentrations [27,28]. For this reason NMR spectra were measured at low protein concentrations and acidic pH. At high detergent/protein molar ratios, the detergent resonances obscured many protein resonances, allowing observation of only three segments of the protein spectrum. These regions are shown in Fig. 6 for suspensions of porcine MBP in aqueous solution and with LPCL; and in Fig. 7 for mixtures of the

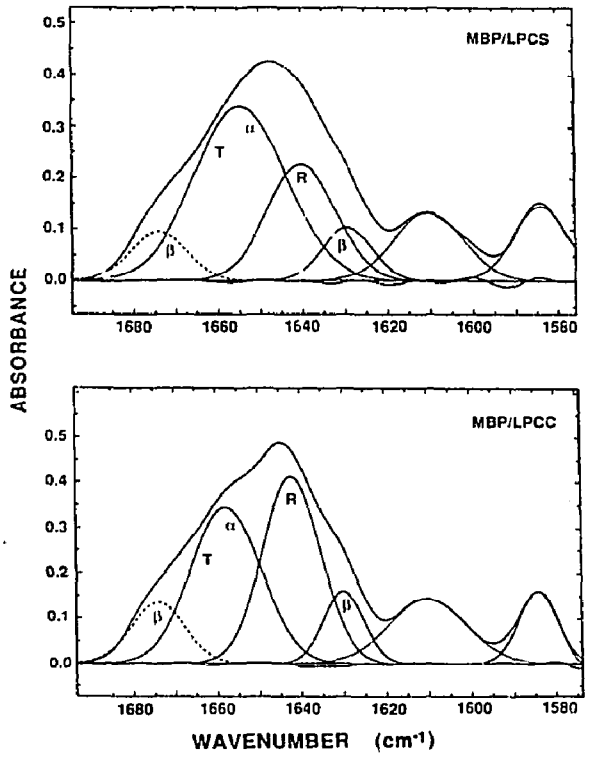

Fig. 5. Amide I region envelopes of the infrared spectra of MBP in LPCS (top) and LPCC (bottom) suspensions at concentrations 1:150 (mM) in 0.05 M phosphate buffer, pH 7, and 298 K. The best-fitted component bands labeled β, T, α and R represent the contributions of β-structures, turns, α-helices and unordered (random) segments of the polypeptide chain. The other bands present are of undefined origin. The lines near the baseline represent the difference between the experimental spectrum and the fitted bands.

protein with LPCC, LPCD, LPCM and LPCS. Compared to the protein spectrum in aqueous solutions, the spectra of MBP in lysophosphatidylcholine suspensions remained generally sharp and well-resolved in the presence of the detergents; although there was selective broadening of some resonances and a number of peaks underwent changes in their spectral position. The broadening of resonances and changes observed in the chemical shifts of peaks assigned to specific residues relative to the values observed in aqueous solutions are summarized in Table 3. The linewidths of the His-ϵ_1-CH peaks of residues 10, 32, and 89, the ϵ_3-CH and ζ_2-CH of Trp-117, the Tyr-δ_1,δ_2-CH and ϵ_1,ϵ_2-CH of residues 128 and 135, and the Met-ϵ-CH_3 of residues 21 and 176, increased with the acyl chain length of the lysolipid in the dispersion (Fig. 7). The chemical shift changes of the His-ϵ_1-CH peaks of residues 10, 32, 89 and 139, measured in suspensions of different detergents showed an increase in magnitude for the lysolipids with longer acyl chains; similar observations were made for the changes in the spectral position of the Met-ϵ-CH_3 resonances of residues 21 and 176. A splitting of Met-21-ϵ-CH_3 was observed in the spectra of the protein associated with LPCD, LPCL, LPCM, LPCP and LPCS micelles.

The effects of temperature on the myelin basic protein/lysophospatidylcholine complexes was investigated by measuring the spectra of the protein bound to detergent micelles at temperatures between 278 and 338 K. As the temperature was raised, gradual decreases in the linewidths of resonances and changes in the spectral position of some lines were observed, suggesting that within this temperature range no sudden transitions occurred in the micellar system. For the detergents with longer acyl chains, LPCL, LPCM, LPCP and LPCS, variations of temperature induced changes in the spectrum of the protein qualitatively similar to those observed for suspensions of detergents of different acyl chain lengths. Thus, for example, in MBP/LPCP mixtures the resonances arising from the ϵ_3-CH and ζ_2-CH of Trp-117 were broadened beyond observation at temperatures between 278 and 298 K, but as the temperature was increased further the doublet

Table 2

Percentage fractional areas of the amide I infrared bands of bovine MBP in several detergens at 298 K and pH 7 in 0.05 *M* phosphate buffer

Detergent	β-Structure	Random	α-Structure and turns	β-Structure
LPCC	21	33	26	20
LPCD	21	32	27	20
LPCL	22	30	29	19
LPCM	22	27	32	19
LPCP	22	22	37	19
LPCS	21	29	30	20
LPC	22	31	26	20

corresponding to the ζ_2-CH proton became visible at 308 K, and both the ϵ_3-CH and ζ_2-CH doublets were observed at 338 K (Fig. 8). Comparison of these results with the protein spectra measured in suspensions of different detergents at room temperature, where a progressive increase in linewidth of the ϵ_3-CH and ζ_2-CH resonances occurred for the lysolipids with longer acyl chains (Fig. 7), showed that like effects were observed on these resonances by either increasing the temperature of the MBP/LPCP complexes or by the formation of complexes of the protein with detergents of acyl chains shorter than palmitoyllysophosphatidylcholine. Similarities between the effects of temperature and of detergents with different hydrocarbon chain lengths were observed also for the lines corresponding to the Tyr-δ_1,δ_2-CH and ϵ_1,ϵ_2-CH of residues 14 and 135, the relative intensities of the two components of the split Met-21-ϵ-CH_3 resonance, and the chemical shift changes of the His-ϵ_1-CH peaks of residues 10, 32, 89 and 139. If the spectral changes observed varying the temperature of the suspensions reflect the extent of the binding of the protein to the detergent micelles, the similarities between the effects of temperature and detergent acyl chain length lead to the conclusion that the protein binds less tightly to the micelles of the lysoplipids of shorter chains.

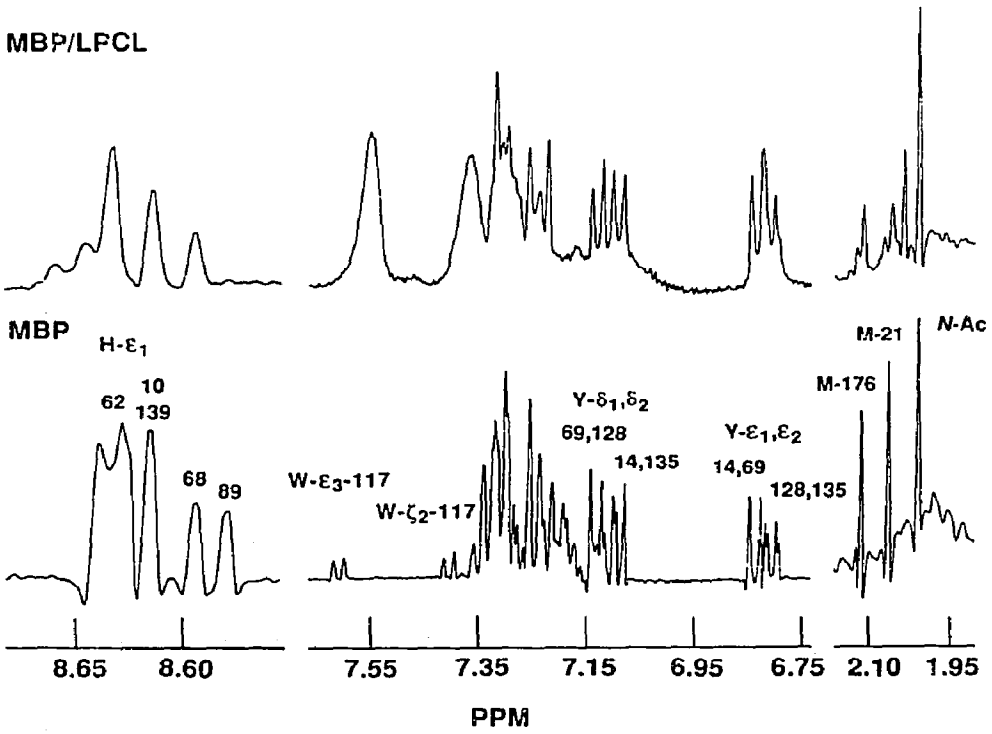

Fig. 6. Three regions of the ^1H NMR spectra of porcine MBP (1 m*M*) in aqueous solutions (bottom) and in LPCL suspensions at [detergent]/[protein] = 150 (top), in ^2H_2O at 298 K, pH 3.0. The letters and the numbers over the resonances in the bottom spectrum indicate the type of residue and the position in the primary structure of MBP, respectively.

Incorporation of deuterated chloroform into detergent/protein complexes induced changes in the spectrum of the basic protein in lysophospahtidylcholine suspensions. Figure 9 shows spectra of MBP in LPCD suspensions to which different amounts of C^2HCl_3 have been added. As the amount of chloroform incorporated into the complexes was increased, downfield shifts of the His-ϵ_1-CH of residues 10, 32, 89 and 139 peaks, coalescence of the Met-21-ϵ-CH_3 spectral lines, and broadening of the Tyr-δ_1,δ_2-CH and ϵ_1,ϵ_2-CH resonances of residues 14 and 135 were observed. These effects resembled those induced in the protein spectrum in suspensions of lysolipids with different acyl chains, as the hydrocarbon chain length of the detergent increased.

3.4 Small-angle X-ray scattering

The scattering spectra of lysophosphatidylcholine mixtures with MBP are similar after subtraction of the water scattering spectrum. There are large, broad peaks which in all probability correspond to diffraction 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 were analyzed using the Guinier approximation [29], and the results are given in Table 4. The radii of gyration (R_g) of lysophosphatidylcholine micelles in the aggregates increased with acyl chain length suggesting a corresponding increase in the average micelle size.

3.5 ^{31}P NMR spectra of protein / detergent complexes

Owing to the strong dependence of the NMR signal of the phosphate groups of phospholipids on the orientation of the head group relative to the applied external magnetic field, ^{31}P NMR has

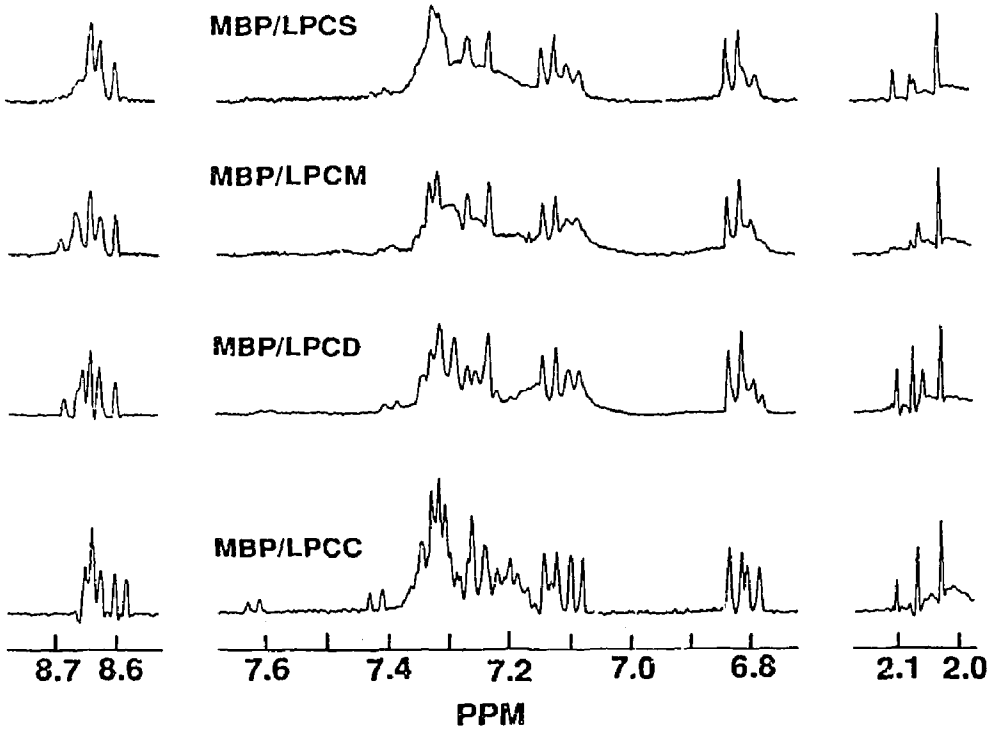

Fig. 7. Three regions of the ^1H NMR spectra of porcine MBP (1 mM) in suspensions of lysophosphatidylcholines with different acyl chain lengths at [detergent]/[protein] = 150, in ^2H_2O at 298 K, pH 3.0 The lysolipids in the suspensions are indicated on the labels above the spectra.

been extensively used to distinguish between phospholipid phases [30]. The spectra of aqueous dispersions of long chain lysophosphatidylcholines in the lamellar phase show broad lines with a width of about 40 ppm, and a characteristic low-field shoulder, arising from the anisotropy of the chemical shift tensor of the phosphate group [31,32]. In the micellar phase combinations of lateral diffusion, micelle rotation, and exchange with free lipid in the solvent, average the ^{31}P chemical shift tensor of the head group phosphate ester and as a result only narrow lines are observed in the spectra [30–32]. The effects of temperature on the myelin basic protein/lysophospatidylcholine complexes was investigated by measuring the ^{31}P spectra of LPCD, LPCL, LPCM, LPCP and LPCS at temperatures between 278 and 318 K. In all cases narrow lines characteristic of the isotropic motion of phosphate groups in lipid micelles were observed.

3.6 ^{13}C NMR relaxation times

The values measured for the spin–lattice relaxation times of the carbon nuclei of LPCD, LPCL and LPCP with or without C^2HCl_3 incorporated into the micelles are given in Table 5. The incorporation of chloroform into the micelles increased the relaxation times of the carbon nuclei of the acyl chain of the detergents, but did not affect significantly the relaxation times of the carbon nuclei of the glycerol backbone or the phosphorylcholine moiety. The T_1 values measured for the carbon nuclei of palmitoyllysophosphatidylcholine in MBP/LPCP complexes and with chloroform or n-hexanol incorporated into

Table 3

Changes in the chemical shifts, $\Delta\delta$[a], of MBP resonances observed in the spectra of detergent solutions relative to the chemical shifts measured in aqueous solutions of myelin basic protein at 298 K and pH 3. The protein-to-detergent molar ratio was 1:150

Resonance	Residue No.	$\Delta\delta \pm 0.008$ (ppm)					
		LPCD	LPCL	LPCM	LPCP	LPCS	LPC
N-Ac-CH_3	–	0.000	0.002	−0.001	0.000	0.003	−0.004
His-ϵ_1-CH	10	0.033	0.035,b [b]	0.040,b	0.050,b	0.035,b	0.025
	23	−0.005	−0.009	−0.011	−0.014	−0.007	−0.009
	26	0.000	0.000	−0.002	−0.005	0.002	0.000
	32	0.012	0.019,b	0.032,b	0.034,b	0.014,b	0.013
	61	−0.005	−0.005	−0.007	−0.007	−0.007	−0.011
	62	0.003	0.004	0.006	0.007	0.007	−0.006
	68	0.001	0.004	0.004	0.005	0.003	0.003
	78	0.000	0.000	0.006	0.011	0.004	0.000
	89	0.110,b	0.120,b	0.140,b	0.150,b	b	0.090,b
	139	0.080	0.085	0.098	0.111	0.090	0.070
Trp-ϵ_3-CH	117	−0.013,b	−0.023,b	b	b	b	0.000,b
Trp-ζ_2-CH	117	−0.025,b	−0.025,b	−0.029,b	b	−0.012,b	−0.007,b
Tyr-δ-CH_2	14	0.009,b	0.009,b	0.009,b	0.009,b	0.009,b	0.006
	69	0.006	0.006	0.007	0.007	0.007	0.004
	128	0.006	0.006	0.007	0.007	0.007	0.004
	135	0.009,b	0.009,b	0.009,b	0.009,b	0.009,b	0.006
Tyr-ϵ-CH_2	14	0.003	0.007	0.007	0.008	0.007	0.004
	69	0.003	0.007	0.007	0.008	0.007	0.004
	128	0.011	0.013	0.019,b	0.013,b	0.010,b	0.007
	135	0.004	0.014	0.019,b	0.014,b	0.014,b	0.014
Met-ϵ-CH_3	21	−0.005,b	−0.005,b	−0.007,b	−0.008,b	−0.002,b	−0.005,b
	–	0.009,b	0.009,b	0.006,b	0.004,b	0.005,b	b
	176	−0.002	−0.004	−0.007,b	−0.010,b	−0.008,b	−0.005,b

[a] Positive values of $\Delta\delta$ indicate downfield shifts and negative values upfield shifts.
[b] b indicates broadening of the resonances.

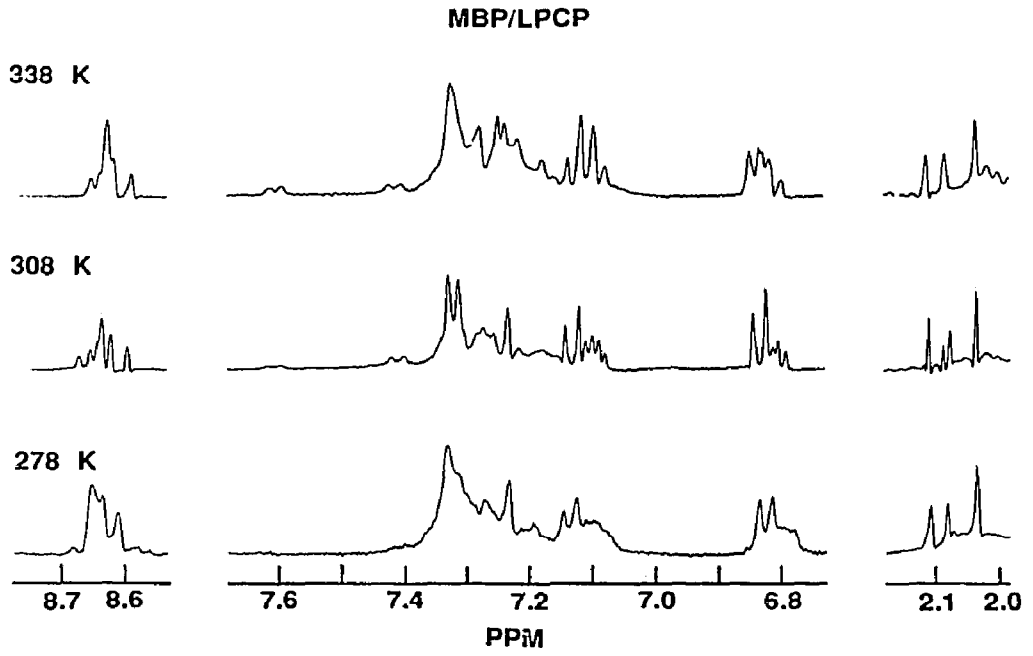

Fig. 8. Three regions of the ^1H NMR spectra of porcine MBP (1 mM) in suspensions of LPCP at [detergent]/[protein] = 120, in ^2H_2O, pH 3.0, at different temperatures. The temperatures are indicated on the left hand side of the spectra.

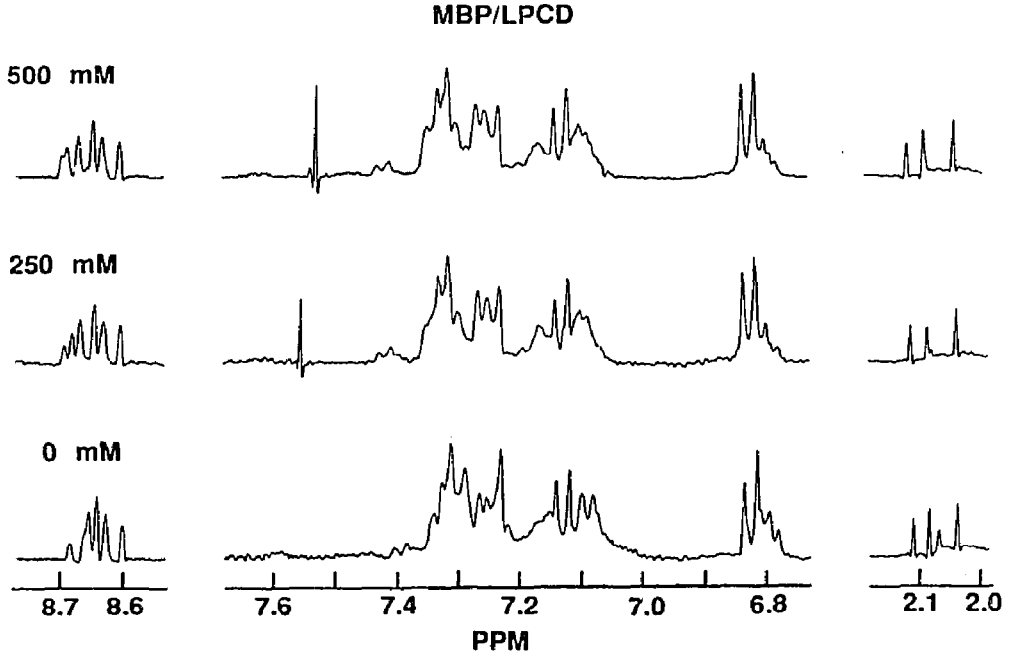

Fig. 9. Three regions of the ^1H NMR spectra of porcine MBP (1 mM) in suspensions of LPCP at [detergent]/[protein] = 120, in ^2H_2O, at 298 K, pH 3.0, with C^2HCl_3 added. The concentrations of chloroform are indicated on the left hand side of the spectra.

Table 4

Calculated [a] acyl chain length and measured average radius of gyration (R_g) of micelles of several lysophosphatidylcholines in complexes with porcine MBP at 298 K and pH 7 in 0.05 *M* phosphate buffer

Detergent	Acyl chain length (nm)	Peak position (nm)	R_g (nm)
LPCC	0.63	None	2.29
LPCD	1.13	3.05	2.66
LPCL	1.38	4.19	3.00
LPCM	1.63	4.26	3.50
LPCS	2.13	5.28	4.40
LPC	–	4.87	4.62

[a] Using the expression given by Tanford [33].

the lysolipid micelles are given in Table 6. Relative to the values obtained for detergent suspensions, the presence of the protein did not change the spin–lattice relaxation times of the lysophosphatidylcholine carbon nuclei. Addition of chloroform or n-hexanol to MBP/LPCP complexes resulted in an increase of the observed relaxation times. The presence of chloroform increased the relaxation times of carbon nuclei in the acyl chain but did not affect the relaxation of the backbone or headgroup carbon nuclei. In contrast, the presence of n-hexanol increased significantly the longitudinal relaxation times of carbon nuclei in the acyl chain, backbone and headgroup.

4. Discussion

CD, FT-IR and ^{1}H NMR data demonstrated that conformational changes occur in myelin basic protein as the result of its interactions with lysophosphatidylcholine micelles. The amount of α-helix induced in the protein molecule depended on the length of the acyl chain of the detergent in the suspension, and reached a maximum value for each lysolipid at sufficiently high detergent/protein molar ratios. The results suggested that there are discrete interaction sites in the protein molecule, and that the participation of different sites in the association with the lysolipids is determined by one or several proper-

Table 5

Longitudinal relaxation times (T_1) of protonated carbon nuclei of lysophosphatidylcholines with and without chloroform incorporated into the micelles. Measurements were carried out in 0.05 *M* detergent suspensions with 43 μl/ml chloroform, at pH 3 and 300 K. T_1's are given in s and the estimated error is ±12%

Nucleus	LPCD	LPCD + C^2HCl_3	LPCL	LPCL + C^2HCl_3	LPCP	LPCP + C^2HCl_3
C_1 [a]	2.568	3.469	2.677	3.670	2.803	4.301
C_2	1.389	2.315	1.324	2.549	1.308	2.787
C_3	0.986	1.648	0.997	2.047	1.045	2.278
C_M [b]	0.521–0.627	0.723–1.151	0.525–0.650	0.858–1.017	0.536–0.781	0.803–1.079
C_{N-1} [c]	0.429	0.552	0.459	0.572	0.462	0.623
C_N [d]	0.505	0.716	0.549	0.683	0.573	0.615
C_{G1} [e]	0.210	0.215	0.212	0.219	0.191	0.211
C_{G2}	0.394	0.386	0.378	0.362	0.345	0.342
C_{G3}	0.245	0.254	0.214	0.238	0.207	0.230
C_{Cl} [f]	0.550	0.527	0.531	0.514	0.512	0.505
C_{C2}	0.548	0.513	0.499	0.506	0.439	0.460
C_{C3}	0.709	0.681	0.637	0.674	0.686	0.668

[a] The numbering of carbon atoms starts with the methyl group at the end of the acyl chain.

[b] C_M refers to the group acyl chain methylene carbon atoms whose resonances are not fully resolved in the spectrum of lysophosphatidylcholines, i.e. 4–7 in LPCD, 4–9 in LPCL and 4–13 in LPCP. The range of values determined for the resonances of these groups of nuclei are given in the table.

[c] C_{N-1} refers to the penultimate methylene carbon atom of the acyl chain, i.e. 8 in LPCD, 10 in LPCL and 14 in LPCP.

[d] C_N refers to the last methylene carbon atom of the acyl chain, i.e. 9 in LPCD, 11 in LPCL and 15 in LPCP.

[e] C_{Gi} refer to the glycerol backbone carbon atoms, C_{G1} is bonded to the acyl chain, and C_{G3} to the phosphorylcholine headgroup.

[f] 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.

ties of the micelles related to the acyl chain length of the detergents.

Micelle shape and size are physicochemical properties which depend on the nature and concentration of detergents, the pH and ionic strength of the aqueous medium, and the temperature of the systems. At constant detergent concentration, pH, ionic strength and temperature, the shape and size of the micelles formed by a given type of detergent such as lysophosphatidylcholines, are determined by the length of their acyl chains [25,33]. The small-angle X-ray scattering data of protein/lysolipid complexes indicated that the size of the detergent micelles in the aggregates increased with acyl chain length (Table 4). Increasing the length of the hydrocarbon chain of the non-charged detergent acyl hexaoxyethylene glycol monoether, or the cationic detergent acyl trimethyl ammonium, from 8 to 16 carbon atoms increases the weight average aggregation number of the micelles formed by nearly three orders of magnitude; the data can only be explained by a change in shape of the micelles from quasi-spherical for the shorter acyl chains to rod-like for chains with more than 14 carbon atoms [34–36]. However, similar increases in the hydrocarbon chain length for zwitterionic *N*-acyl betaine detergents increased the micelle size, but did not affect their shape [37]. Differential scanning calorimetric measurements show that aqueous dispersions of palmitoyllysophosphatidylcholine undergo a gel-to-liquid crystalline transition at 276 K [38]; and ^{31}P NMR spectra measured at 298 K indicate that the detergent forms micelles at this temperature [31]. Differential scanning calorimetry, quasi-elastic laser scattering, ^{31}P NMR and Raman spectroscopy demonstrated that aqueous dispersions of stearoyllysophosphatidylcholine in 0.05 *M* KCl can undergo a sharp lamellar to micellar transition at 299.2 K [32]. Correlated data from diffusion, viscosity and sedimentation measurements indicate that egg yolk lysophosphatidylcholine suspensions, comprising a mixture rich in longer acyl chain species, form spheroid micelles with an axial ratio of approximately 4 [39]. The data from NMR measurements of myelin basic protein/lysophosphatidylcholine complexes at different temperatures suggested that under the experimental conditions employed, increases in the hydrocarbon chain length of the zwitterionic lysophosphatidylcholines did not result in a change of lysolipid phase or micellar shape. Thus, in the temperature range 278 to 318 K, under the experimental conditions employed and in the presence of MBP, all the detergents studied appear to form spheroid micelles whose average size depend of the length of the acyl chain. This interpretation is in agreement with the conclusions of studies of the physicochemical characteristics of complexes of myelin basic protein with dodecylphosphocholine and mixed dodecylphosphocholine/palmitoyllysophosphatidic acid micelles [10,12].

Values of the secondary structures induced in the polypeptide chain by the interactions with lysolipid micelles measured by CD and FT-IR spectroscopy are given in Tables 1 and 2. The differences in the values estimated for the α-helical content of the protein by both experimental techniques are shown to be only apparent when an estimate of the percentage of the polypeptide chain involved in turns is obtained and subtracted from the values calculated using FT-IR spectroscopy. The content of turns in the protein molecule from analyses of the amide I region of the FT-IR spectra of MBP free in solution and interacting with dimyristoylphosphatidylglycerol bilayers yielded values of 16% and 15%, respectively [17]. Subtracting any of these percentages from those calculated for α-structures and turns in Table 2, yielded values for α-helix contents that are in good agreement with those of Table 1, with the exception of the percentage for suspensions of the basic protein in LPCC, which seem to be overestimated in the FT-IR analyses. The discrepancies regarding the amounts of β-structure and non-ordered conformations obtained from CD (Table 1) and FT-IR (Table 2) analyses are evident, and they reflect, at least in part, the high degree of inaccuracy of the CD calculations of nonordered regions of the polypeptide backbone. Comparison of the quantitative estimation of the secondary structure of 13 globular proteins by FT-IR spectroscopy, CD and X-ray crystallography shows good agreement in the percentage of α-helix determined by the three methods, but for

eight proteins the content of β-structure calculated by CD underestimated by values between 15 and 30% the amounts obtained employing the other two techniques [16]. In complexes with dimyristoylphosphatidylglycerol bilayers the major ordered conformation adopted by MBP is a β-structure (53%); the fractional areas corresponding to α-helical conformations, unordered segments of the protein, and different types of turns of the polypeptide chain were 15%, 7% and 15%, respectively [17]. These considerations suggest that the β-structure content of MBP/lysophosphatidylcholine complexes may have been underestimated in the analyses of CD measurements. Alternatively, the higher protein concentrations employed in the FT-IR measurements may have induced the formation of larger amounts of β-structures [28].

For lysophosphatidylcholines with acyl chains between 6 and 16 carbon atoms a correlation was found between the α-helix induced in the protein and the length of the hydrocarbon chain of the lysolipids; the helical content of the polypeptide increased with the length of the chain of the detergent in the suspension. For detergents with chains longer than 16 carbon atoms (LPCS and LPCO) and for mixtures of lysolipids with acyl chains of 16 and 18 carbon atoms (LPC), the helical content decreased relative to the one measured for LPCP (Tables 1 and 2; Figs. 6 and 7). Since in these systems the acyl chain length characterized the size of spheroid micelles, an explanation for the existence of an average micelle size that maximized the binding of the protein could be that intramolecular long-range interactions arising from tertiary structures in MBP limit the number of regions that could form helices and their relative spatial configuration. This hypothesis was proposed to explain the interactions of MBP and sequential peptides obtained by cleavage of the protein molecule with mixed neutral–anionic detergent micelles [11]. Intramolecular interacions may prevent the extra folding of the polypeptide chain that would be required to bring the protein regions capable of forming helices into proximity of micelles smaller than those formed by LPCP; in addition, the smaller micelles may not provide sufficient space to accomodate the helices that MBP is capable of forming in associations with lysophosphatidylcholines. For micelles larger than those formed by LPCP, the smaller curvature of their surface may demand an unfolding of the protein unfavourable to the long-range intramolecular interactions. Evidence supporting this interpretation are the substantial portions of the protein molecule that form α- and β-structures in the interactions with lysophosphatidylcholines (Table 2), and the small amount of binding of MBP to phosphatidylcholine vesicles [3,40–43].

The effect of fluidity on the interactions of the protein with lysophosphatidylcholines was studied by incorporating chloroform into the hydrophobic core of the detergent micelles. The fluidity of an ensemble of lipid molecules is related to the average molecular motion of the acyl chains of the lipid molecules [44]. A motion profile for the hydrocarbon chains can be determined by measuring the motion of their relaxation times [30]. The values of the ^{13}C spin–lattice relaxation times, T_1, of the different carbon atoms in the acyl chains was used as a measure of the fluidity of the lysophosphatidylcholine micelles. Incorporation of chloroform into the micelles increased the relaxation times of carbon nuclei of the hydrocarbon chains indicating an increase in the fluidity of the micelles with and without MBP (Tables 5 and 6). CD and ^1H-NMR spectroscopy showed that for lysolipids with acyl chains of 10–14 carbon atoms, incorporation of chloroform increased the helical content of the polypeptide and produced changes in the spectrum of the protein which were characteristic of the interactions with longer chain detergents (Figs. 3 and 9); addition of $CHCl_3$ did not change the α-helicity of the protein molecule in MBP/LPCS suspensions (Fig. 3). The data suggested that the conformational changes induced in the protein molecules were enhanced by interactions with more fluid detergent micelles, but the extent of these changes was limited by micellar size as was observed for the case of the less fluid lysophosphatidylcholine micelles without chloroform.

Further investigation of the effects of micelle size and fluidity on the interactions of the protein with lysophosphatidylcholines was carried out by

incorporating methanol and n-hexanol into the hydrophobic core of the detergent micelles. Acyl alcohols induce a biphasic effect on the phase transition temperature (T_m) of saturated phosphatidylcholine bilayers. At low concentrations there is a decrease in T_m with increasing alcohol concentration, whereas at higher concentrations there is an abrupt reversal and T_m increases with increasing alcohol concentration [45,46]. For the case of ethanol the effects on the thermotropic properties of the bilayers depend systematically on the length of the lipid hydrocarbon chain [46]. The reversal of T_m is a consequence of the induction of an unusual gel phase in which the lipid molecules from opposing monolayers interdigitate reducing the bilayer thickness [47]. This interdigitated phase is induced in a manner similar to that of other surface active small molecules such as benzyl alcohol, methanol, phenylbutanol, and glycerol [48], where the amphiphilic molecules anchor at the interfaces by virtue of their polar moiety, with the non-polar part of the molecule intercalating between the acyl chains. Since for these small molecules the non-polar moieties are short compared to the lipid hydrocarbon chains, the interfacial location would potentially cause voids between chains in the bilayer interior. Because the energy of formation of holes in hydrocarbon liquids is extremely large, the lipid acyl chains interdigitate to eliminate the formation of voids [49]. The influence of a series on n-acyl alcohols on gramicidin channel lifetime in monoolein–squalene membranes has been interpreted as the differential effect of long and short chain alcohols on bilayer thickness [50,51]. Addition of methanol and n-hexanol to myelin basic protein–lysolipid suspensions resulted in decreases of the helical content of the polypeptide chain of the protein bound to detergent micelles, although the incorporation of n-hexanol increased the fluidity of the lysophosphatidylcholine micelles (Table 6). Similar interpretations may explain the effects of methanol and n-hexanol on the protein/detergent complexes and the effects of short chain alcohols on lipid bilayers. Methanol would remain mostly at the micellar interface owing to its water–hydrocarbon partition coefficient, and consequently have only a small effect on the hydrocarbon core. The alcohol group of n-hexanol would remain close to the water interface whilst its acyl chain would intercalate between the lysolipid hydrocarbon chains. The changes observed in the motion of the head groups, glycerol backbones and acyl chains of MBP/LPCP complexes (Table 6) support this interpretation. The different lengths would require that the detergent chains fold more than in the absence of the n-alcohol to prevent the formation of voids in the micelle core. The extra folding of the chains would diminish the overall

Table 6

Longitudinal relaxation times of protonated carbon nuclei of palmitoyllysophosphatidylcholine in 100:1 molar ratio protein–detergent suspensions with 0.05 *M* lysolipid and 43 μl/ml chloroform or n-hexanol. Measurements were carried out at pH 3 and 300 K. T_1's are given in s and the estimated error is ±12%

Nucleus	MBP/LPCP	MBP/LPCP + C^2HCl_3	MBP/LPCP + n-Hexanol
C_1 [a]	2.865	3.813	3.518
C_2	1.251	2.881	2.198
C_3	0.985	1.435	1.946
C_M [b]	0.506–0.601	0.662–0.784	1.584–1.946
C_{N-1} [c]	0.465	0.470	–
C_N [d]	0.535	0.582	0.595
C_{G1} [e]	0.177	0.191	0.278
C_{G2}	0.335	0.387	0.459
C_{G3}	0.216	0.237	0.575
C_{C1} [f]	0.495	0.483	0.597
C_{C2}	0.440	0.456	0.575
C_{C3}	0.663	0.680	0.724

[a] The numbering of carbon atoms starts with the methyl group at the end of the acyl chain.

[b] C_M refers to the group acyl chain methylene carbon atoms whose resonances are not fully resolved in the spectrum of palmitoyl lysophosphatidylcholine, i.e. 4–13 in LPCP. The range of values determined for the resonances of these groups of nuclei are given in the table.

[c] C_{N-1} refers to the penultimate methylene carbon atom of the acyl chain, i.e. 8 in LPCD, 10 in LPCL and 14 in LPCP.

[d] C_N refers to the last methylene carbon atom of the acyl chain, i.e. 9 in LPCD, 11 in LPCL and 15 in LPCP.

[e] C_{Gi} refer to the glycerol backbone carbon atoms, C_{G1} is bonded to the acyl chain, and C_{G3} to the phosphorylcholine headgroup.

[f] 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 ammoniunm carbon atoms.

micelle size and result in a decrease of the α-helix content of the protein, notwithstanding the increase in micelle fluidity. This interpretation is supported by the observation that the effect is less pronounced for the detergents with shorter acyl chains (Fig. 4), which would require a lesser readjustment of the acyl chains and consequently a smaller change in micelle size.

The sequences comprising residues 13–28, 35–45, 58–73, 87–97, 108–119 and 140–159 of the MBP molecule have been identified as sites of possible amphipathic helices (Table IV, ref. [11]). Excluding the residues that would be located at the boundaries, each region comprises a potential eight-residue helix. In addition, shorter amphipathic helices of five, four and five residues could be formed in segments 13–28, 58–73 and 140–159, respectively. The number of residues corresponding to the mol percentage of α-helix measured by CD in each detergent suspension is given in Table 1.

Comparison of the number of residues included in helices (Table 1) with the possible number of residues in amphipathic helices showed that not all potential helices would be realized even in the interactions with LPCP micelles. Considering the number of residues in helical conformations together with the ^{1}H NMR data on changes in chemical shifts of several residues (Table 3), a broad outline of possible locations of the helices formed in the interactions with each lysolipid can be delineated. This does not require in each case that all the residues in a given segment be involved in forming helices. Since the helix content of the protein in LPCC suspensions was only marginally larger than that measured in aqueous solutions, the results obtained with this detergent are left out from the following discussion. The spectral changes measured in the resonances arising from His-10, Tyr-14, Met-21, His-23 and His-89 suggested that regions 13–28 and 87–97 participated in the interactions with all the detergents. The line-broadening of the Trp-117 ϵ_3-CH and ζ_2-CH resonances suggested that segment 108–119 was involved in interactions with LPCM LPCP and LPCS micelles. The little effects observed in the spectral position of His-62 and His-68 indicated that the sequence 58–73 probably was not included in regions of the protein that associated with lysophosphatidylcholine micelles. To satisfy the number of residues in helical conformations in LPCM and LPCP suspensions, at least another region of MBP besides segments 13–28, 87–97 and 108–119, must be involved in the interactions. There were no specific markers for segments 35–45 and 140–159, but the presence of detergents did affect the spectral positions of His-32 and His-139, leaving open the possibility that either segment may also be involved in protein–detergent interactions.

The CD, FT-IR and NMR data on complexes of myelin basic protein with lysophosphatidylcholines acyl chain lengths from 6 to 18 carbon atoms and incorporating chloroform, methanol or n-hexanol suggested that the amount of α-helix induced in the protein molecule depended on the size and fluidity of the micelles in the suspensions, the optimum size corresponding to LPCP micelles. It is unlikely that a similar relationship to the size of the hydrocarbon chains would be found in the binding of MBP to lipid bilayers, but the considerable amounts of α- and β-structures induced in the polypeptide chain by the associations with the detergents, resembled the effects observed for complexes with dimyristoylphosphatidylglycerol bilayers [17]. The size of the micelles limited the participation of different sites of the protein molecule in the interactions, and this dependence allowed the delineation of the protein regions that are more likely to be involved in hydrophobic interactions with bilayers.

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