Interactions of Myelin Basic Protein with Mixed Dodecylphosphocholine/Palmitoyllysophosphatidic Acid Micelles†

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ABSTRACT: The interactions of myelin basic protein and peptides derived from it with detergent micelles of lysophosphatidylglycerol, lysophosphatidylserine, palmitoyllysophosphatidic acid, and sodium lauryl sulfate, and with mixed micelles of the neutral detergent dodecylphosphocholine and the negatively charged detergent palmitoyllysophosphatidic acid, were investigated by ^{1}H NMR spectroscopy and circular dichroic spectropolarimetry. The results with single detergents suggested that there are discrete interaction sites in the protein molecule for neutral and anionic detergent micelles and that at least some of these sites are different for each type of detergent. The data on the binding of the protein and peptides to mixed detergent micelles suggested that intramolecular interactions in the intact protein and in one of the longer peptides limited the formation of helices and also that a balance between hydrophobic and ionic forces is achieved in the interactions of the peptides with the detergents. At high detergent/protein molar ratios, hydrophobic interactions appeared to be favored.

The interactions of myelin basic protein (MBP) with membrane lipids is considered to be one of the essential factors contributing to the stability and function of myelin membranes in the central nervous system of many animal species (Boggs et al., 1982a). The basic character of the protein, the relative abundance of negatively charged lipids in myelin membranes where the ratio neutral/anionic lipids is approximately 4:1, and the asymmetric distribution of myelin lipids (Kischner et al., 1984) led to the recognition that binding of MBP to negatively charged lipids could have a central role in the molecular architecture of myelin. For many years the interactions of MBP with anionic lipids (Anthony & Moscarello, 1971; Demel et al., 1973; Boggs et al., 1977) and detergents (Jones & Rumsby, 1977; Smith & McDonald, 1979) have been the focus of active research. Binding of the protein to negatively charged lipids and detergents involves more than Coulombic forces; hydrophobic interactions have been demonstrated to become very important at high lipid/protein ratios (Vadas et al., 1981; Boggs et al., 1982b; Surewicz et al., 1987). A natural progression to these studies would be to investigate the interactions of MBP with mixtures of neutral and anionic lipids or detergents.

The conformational changes induced in MBP by the interaction with anionic and mixed neutral/anionic detergent micelles have been studied by employing ^{1}H NMR and circular dichroism spectroscopy. The information obtained from circular dichroic experiments on the conformational changes induced in protein molecules bound to micelles of different detergents complemented the detailed information provided by high-resolution NMR techniques on the effects of the interactions on specific protein regions. These investigations permitted the identification of protein segments involved in the interactions with negatively charged detergents, and the clarification of the relationship between the effects of ionic and hydrophobic interactions on MBP bound to mixed detergent micelles.

Materials and Methods

Porcine MBP (p-MBP) was kindly provided by Dr. Max Marsh of Eli Lilly & Co. Rabbit MBP (r-MBP) and bovine MBP (b-MBP) were purified according to the method of Law et al. (1984). Peptides were prepared from rabbit MBP by cleavage with porcine pepsin (EC 3.4.23.1) or human thrombin (EC 3.4.21.5) and purified as previously described (Martenson et al., 1981a,b; Law et al., 1984). The purity of the peptides was determined by amino acid analysis, tryptic peptide mapping, and gel electrophoresis at acid pH in 8 M urea. The peptides appeared to be virtually homogeneous by all these objective criteria. The numbering of residues in the protein sequence is the same as employed in Figure 8 of Mendz et al. (1984). This convention was adopted to maximize the homologies in the primary structure of proteins derived from different species. Palmitoyllysophosphatidic acid, (LPAP) was obtained from Serdary Research Laboratories Inc. (London, Ontario), and lysophosphatidylglycerol (LPG), lysophosphatidylserine (LPS), and sodium lauryl sulfate (SDS) were from Sigma Chemical Co. (St. Louis, Missouri). The fatty acid spin-labels 5-doxylstearate [2-(3-carboxypropyl)-2-tridecyl-4,4-dimethyl-3-oxazolidinyloxy], 12-doxylstearate, and 16-doxylstearate were purchased from Aldrich Chemical Co (Milwaukee, WI). Dodecylphosphocholine (DPC) was synthesized by the method described by Brown (1979).

The proteins and peptides were lyophilized twice from 99.5% ^2H_2O and dissolved in 99.96% ^2H_2O (Merck Sharp & Dohme, Montreal) at concentrations between 0.50 and 1.0 mM in 5-mm o.d. precision NMR tubes (Wilmad, Buena, NJ). The pH was adjusted with ^{2}HCl or NaO^{2}H and measured with an Ingold 6030-02 microelectrode, values being reported as meter readings. Increasing amounts of DPC/LPAP mixtures were added to the protein solutions to obtain a series of detergent/protein molar ratios from 0 to 240.

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The ^1H NMR measurements were made with a Bruker WM 400 spectrometer. Spectra were recorded at 298 K by accumulating 1000–2000 transients over 16K data points. A radiofrequency pulse of 90° (7–9 μs) was used with a spectral width of 4000 Hz and a repetition time of 2.10 s. To avoid dynamic range problems in measuring protein spectra, the detergent resonances corresponding to the choline methyl protons and to methyl and methylene protons of the acyl chain 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-4-silapentane-1-sulfonate) by the method previously described (Mendz et al., 1982). The exchange with ^2H_2O of −NH and $-NH_2$ groups in the protein/lipid system was followed by lyophilizing a sample of MBP with DPC/LPAP from H_2O, suspending the mixture in ^2H_2O, and measuring the NMR spectrum every 10 min (with 256 scans).

Samples containing spin-labels were prepared by adding a measured amount of free radical in ethanol to a protein/detergent aqueous suspension to obtain the final composition desired, lyophilizing the mixture, and dissolving it again in ^2H_2O.

Circular dichroic (CD) spectra were measured at 295 K with a JEOL-500C spectropolarimeter at various detergent/protein ratios. Spectral range was 195–275 mm, 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. Protein concentration was 8×10^{-6} M, and the concentrations of peptides, 5.6×10^{-5} M; detergent concentrations ranged from 4×10^{-5} to $8.0\ 10^{-3}$ M. Measurements were carried out in 0.05 M phosphate buffer, pH 7.0. The CD spectra of detergents alone were negligible over the spectral range recorded. In the range of concentrations employed, the mixed detergent/protein suspensions were optically clear. A small degree of turbidity was observed in protein suspensions with either LPAP or SDS at the higher detergent concentrations. The α-helix content was calculated by fitting the CD spectrum to a weighted average of values for 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 hydrophobicity of r-MBP and peptides was calculated according to the method of Hopp and Woods (1983). The procedure employs a hydrophilicity index given to each type of residue, e.g., 3.0 to R, −3.4 to W, etc., and averages their value over a sequence. Positive values of the average indicate a hydrophilic segment and negative ones a hydrophobic segment. Regions of the protein with a potential for forming amphipathic helices were identified by constructing helical wheels (Schiffer & Edmundson, 1967; Randall & Zand, 1985) and space-filling models.

Results

Circular Dichroism. CD spectra of bovine MBP were measured in phosphate buffer with increasing concentrations of LPG, LPS, LPAP, or SDS and mixtures of DPC with these anionic detergents up to detergent/protein molar ratios of 200:1. Conformational changes occurred in the protein interacting with detergent or mixed detergent micelles. Table I shows the mole percentage α-helix induced in b-MBP in suspensions of the anionic detergents and neutral/anionic detergent mixtures. The α-helical content of the basic protein in phosphate buffer was approximately 4%. Figure 1 shows the calculated percentage of α-helicity induced in MBP by LPAP/DPC mixtures at anionic/neutral detergent molar ratios from 1:0 to 1:9. At low detergent/protein ratios the induced α-helical content depended on the composition of the detergent mixture; at high detergent/protein molar ratios the protein adopted a maximum of approximately 20% α-helix in all the DPC/LPAP mixtures used. Up to a detergent/protein ratio of 40:1, the richer the mixture in palmitoyllysophosphatidic acid, the greater was the helicity induced in the protein. In the presence of LPAP micelles the maximum helix content measured was 40%; increasing the content of DPC in the mixture of detergents decreased the helicity of the protein. Qualitatively similar effects on the conformation of MBP were observed with mixtures of dodecylphosphocholine and sodium lauryl sulfate. The maximum helical content observed in the protein by interaction with SDS was 30% at a detergent/protein molar ratio 40:1, corresponding to an SDS concentration of 0.32×10^{-3} M. At higher SDS concentrations aggregates precipitated in the solution under the experimental conditions employed. It was possible, however, to make MBP/DPC/SDS suspensions of molar ratios 1:100:100. The α-helix induced in the protein in DPC/SDS suspensions at detergent/protein concentrations above 50:1 was 19–23%, depending on the detergent mixture. In suspensions of MBP with the other charged detergents, the induced α-helicity in the protein was in each case greater for the interactions with micelles of the anionic detergent than for the interactions with mixed neutral/charged detergent micelles (Table I).

Table I: Mole Percent of α-Helix of Bovine MBP in Several Detergents and Detergent Mixtures at 295 K and pH 7 in 0.05 M Phosphate Buffer[a]

detergent	mol % of α-helix	detergent	mol % of α-helix
DPC	18.5[b]	LPAP	40.6
LPG	24.6	LPAP/DPC	20.4
LPG/DPC	19.4	SDS	~33
LPS	22.7	SDS/DPC	22.5
LPS/DPC	21.0		

[a] The molar ratio in the mixtures of dodecylphosphocholine with anionic detergents was 4:1. The molar ratio detergent/protein was 200:1, with the exception of LPAP/MBP and SDS/MBP suspensions. Insoluble aggregates formed at a LPAP/MBP ratio of 70:1. At an SDS/MBP molar ratio of 40:1, the α-helicity induced was 30% and insoluble detergent/protein aggregates formed. The value given in the table for the α-helicity of MBP in SDS suspensions was an estimate derived from the α-helix content measured by titrating protein solutions. [b] From Mendz et al. (1984).

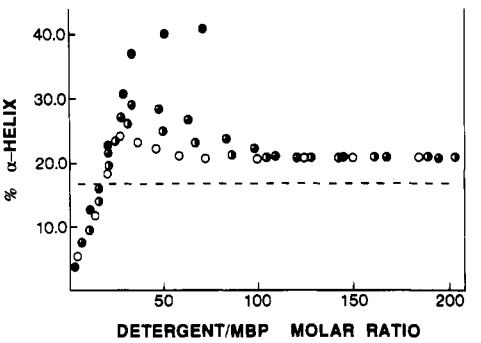

FIGURE 1: Calculated percent of α-helicities of bovine myelin basic protein as a function of detergent/protein ratio at 295 K, in 0.05 M phosphate buffer, pH 7. The LPAP/DPC detergent mixtures employed had the following molar ratios: (●) 1:0, (◕) 1:3, (◑) 1:6, and (○) 1:9. The horizontal dashed line indicates the maximum value measured for solutions in DPC (Mendz et al., 1984).

Table II: Mole Percent of α-Helix and Number of Residues in Helical Conformations in r-MBP and Peptides Derived from It in the Presence of DPC/LPAP Micelles, Measured at 295 K and pH 7 in 0.05 M Phosphate Buffer[a]

	mol % of α-helix			no. of residues		
peptide	H_2O[b]	DPC[b]	DPC/LPAP	H_2O[b]	DPC[b]	DPC/LPAP
1–44	8.9	9.5	12.9	4	4	6
45–90	11.5	13.5	31.1	5	6	13
1–90	8.6	15.8	18.2	7	14	16
91–179	4.0	9.6	14.0	3	8	11
1–98	11.8	28.8	38.6	11	28	37
99–179	4.4	16.0	41.5	3	11	30
1–179	4.0	18.5	20.4	7	31	34

[a] The molar ratio of the peptide/DPC/LPAP mixtures was 1:120:30. [b] From Mendz et al. (1984).

These observations suggested that the conformational changes induced in MBP by interaction with LPAP or SDS were at least partially different from the changes induced by interaction with DPC micelles. At high detergent/protein ratios the interactions with mixed detergent micelles induced changes in the conformation of the protein that could not be regarded as the "addition" of the changes brought about by each type of detergent. The observed α-helicity yielded values closer to those measured for the neutral detergent.

The calculated percent of α-helix measured for several peptides derived from rabbit MBP in suspensions of mixed DPC/LPAP micelles at detergent/peptide molar ratios of 150:1 are given in Table II. For comparison the values previously obtained for water solutions and in the presence of DPC micelles (Mendz et al., 1984) are also given in the table. The helix induced in the peptide molecules was higher in suspensions with mixed DPC/LPAP micelles than with DPC micelles; in particular, the increase in α-helicity measured for peptides 45–90 and 99–179 was greater than 2-fold (Table II).

Proton Nuclear Magnetic Resonance of Protein/Detergent Complexes. At neutral pH myelin basic protein aggregates even at low concentrations (Chapman & Moore, 1978; Smith, 1985). For this reason the spectra reported here have been measured at low protein concentrations and acidic pH. At high nondeuterated 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 Figure 2 for suspensions of p-MBP with LPG, LPS, and SDS; different protein spectra resulted from the interaction with the micelles of each detergent. The resonances in the spectra of MBP in the three detergent suspensions are broader than the resonances in the protein spectrum in aqueous solutions. In particular, it is possible to observe an increase in line width of the histidyl-ϵ_1-CH peaks between 8.6 and 8.8 ppm, the tyrosyl-ϵ-CH_2 peaks about 6.8 ppm, and the methyl resonances of M-21 and M-176 between 2.0 and 2.1 ppm. Broadening of the aromatic histidyl resonances is stronger in SDS than in LPG or LPS, and the changes in chemical shifts of these peaks are different in the spectra of the three detergents. The increase in line width of the Trp-ζ_2-CH and Trp-ϵ_3-CH resonances, between 7.4 and 7.6 ppm, made them unobservable in the spectra of MBP in LPS and SDS, but these peaks remain visible as broad features in the protein spectrum in LPG. The M-176-ϵ-CH_3 peak is narrow in the MBP spectra in LPG and SDS suspensions of the protein, but not in LPS suspensions. Figure 3 shows two regions of the ^{1}H NMR spectra of MBP in aqueous solution, in aqueous suspension with DPC, and in aqueous suspension with LPAP. One region comprises the resonances of the aromatic ϵ_1-CH histidyl protons, and the other region corresponds to the aliphatic resonances of the methyl groups of

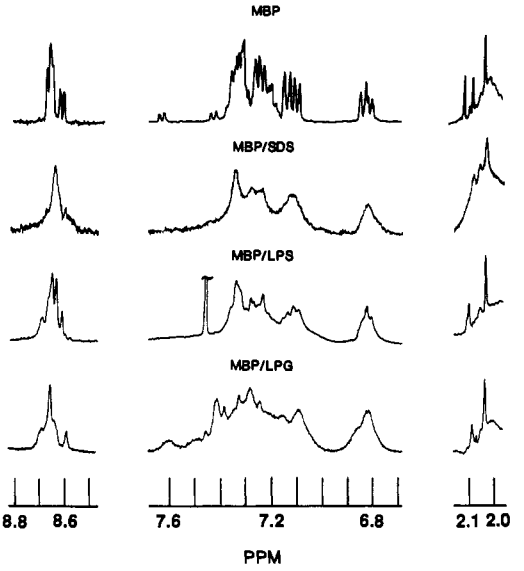

FIGURE 2: Three regions of the ^{1}H NMR spectrum of porcine myelin basic protein (0.5 mM) in aqueous (^2H_2O) solution and in LPG, LPS, and SDS suspensions at 298 K, pH 4. The detergent concentrations were 100, 70, and and 20 mM, respectively. The three regions are shown in the same scale; the interval between markings is 0.1 ppm.

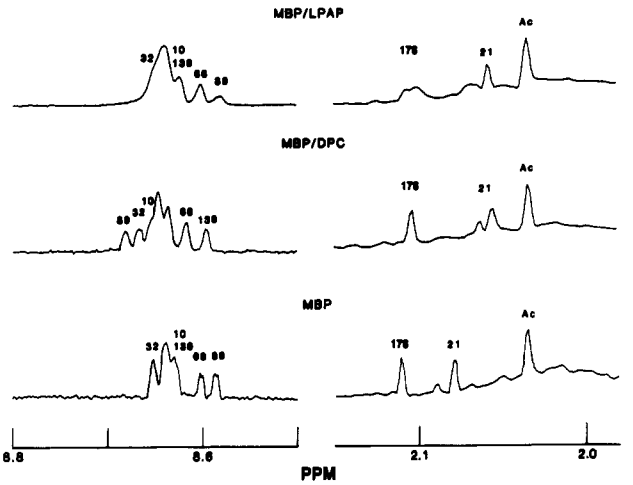

FIGURE 3: Aromatic ϵ_1-histidyl and aliphatic ϵ-methionyl regions of the ^{1}H NMR spectrum of porcine MBP (1.0 mM) in aqueous (^2H_2O) and detergent solutions of DPC and LPAP at 298 K, pH 2.9. The detergent concentrations were 200 mM for DPC and 20 mM for LPAP. The numbers over the resonances in the spectra indicate the residue number in primary structure of MBP.

methionyls 21 and 176, and the acetyl moiety at the beginning of the protein sequence. These resonances arise from residues located throughout the primary structure of MBP: *N*-acetyl, H-10, M-21, H-32, H-68, H-89, H-139, and M-176 (Mendz et al., 1982, 1983a,b, 1986). Although many of the observable resonances from the protein were not greatly affected by the presence of the neutral or the anionic detergents, changes in chemical shift and/or broadening were observed in other resonances, indicating that changes occurred at specific locations in the protein. The changes were similar for both detergents in the case of some resonances, for instance, H-68-ϵ_1-CH, a small downfield shift, and M-21-ϵ-CH, a medium upfield shift and a strong broadening. However, the changes in chemical shift and/or broadening observed for other resonances were different in the presence of DPC or LPAP. For example, the ϵ_1-CH resonance of H-89 underwent a downfield shift 0.88 ppm in the presence of DPC, but it is only broadened in the interaction of MBP with LPAP micelles; in the case of the M-176-ϵ-CH resonance, the interaction of MBP with DPC

Table III: Changes in the Chemical Shifts, $\Delta\delta$, of Resonances Observed in the Spectra of Detergent Solutions Relative to the Chemical Shifts Measured in Aqueous Solutions of Myelin Basic Protein and Peptides Derived from It, at 298 K and pH 2.7[a]

		$\Delta\delta \pm 0.008$ (ppm)					
resonance	residue no.	1–44	45–90	1–98	99–179	r-MBP	p-MBP
N-Ac-CH_3		−0.024		−0.029		−0.027	−0.030
His-ϵ_1-CH	10	+0.007		+0.009		+0.010	+0.009
	23	−0.020		−0.019		−0.019	−0.017
	26	−0.027		−0.002		−0.005	−0.008
	32	−0.002		−0.003		−0.001	−0.013, b
	61		−0.022	−0.029		−0.011	−0.027
	62						−0.018
	68		+0.002	+0.001		−0.013	+0.003
	78		−0.006	−0.002		−0.001	−0.008
	89		+0.053	+0.084		+0.088	+0.080, b
	139				+0.008	+0.007	−0.027
Trp-ϵ_3-CH	117				−0.024, b	b	b
Trp-ζ_2-CH	117				−0.029, b	b	b
Tyr-δ-CH_2	14	−0.011, b		−0.011		−0.011, b	−0.011
	69		−0.051, b	−0.031		−0.049, b	−0.027
	128				−0.074, b	−0.069, b	−0.004
	135				−0.073, b	−0.074, b	−0.027
Tyr-ϵ-CH_2	14	−0.011, b		−0.010		−0.020	−0.027
	69		−0.040, b	−0.033		−0.027	−0.027
	128				−0.018, b	−0.004, b	−0.016
	135				−0.054, b	−0.030, b	−0.009
Met-ϵ-CH_3	21	−0.037, b		−0.023		−0.026, b	−0.039, b
	176				−0.025	−0.033, b	−0.033, b

[a] DPC and LPAP were mixed at a molar ratio of 4:1, and the detergent/protein molar ratio was 240:1. Positive values of $\Delta\delta$ indicate downfield shifts and negative values upfield shifts; b indicates broadening of the resonances.

micelles resulted in a small upfield shift, but in the presence of LPAP this resonance experienced a splitting which appeared as a strong broadening of the peak. These changes could be followed in the spectra of the protein measured in suspensions with different detergent mixtures (Figure 4). Binding of MBP to DPC micelles induced upfield shifts on the methyl resonances of M-21 and M-176 and a splitting in the M-21-ϵ-CH_3 resonance (Figure 4, spectrum at LPAP/DPC, 20:0). The spectra at 20 mM LPAP and 0, 20, and 40 mM DPC show that addition of the neutral detergent to the LPAP/MBP suspension resulted in a downfield shift in the methyl resonance of M-21 and a narrowing of the M-176-ϵ-CH_3 peak. Titration of the LPAP/DPC/MBP, 20:40:1, suspension with LPAP to equimolar amounts of both detergents broadened the M-21 and M-176 methyl resonances, a characteristic effect of the negatively charged detergent (Figure 4, spectrum at LPAP/DPC, 40:40). At a DPC/LPAP ratio of 100:40 the predominant features were typical of the changes induced by the neutral detergent, that is, a splitting of the M-21-ϵ-CH_3 peak and an upfield shift of the M-176-ϵ-CH_3 resonance. Similar observations were made for histidyl-ϵ_1-CH proton resonances.

The resonances in the spectrum of MBP that were not obscured by detergent resonances after titration with (4:1) DPC/LPAP mixtures at a detergent/protein molar ratio of 240:1 remained, with the exception of resonances from tryptophan and tyrosine residues, generally sharp and well resolved in the presence of mixed detergent micelles. The changes observed in the chemical shifts of peaks assigned to specific residues (Mendz et al., 1982, 1983a,b, 1986) in the spectra of p-MBP, r-MBP, and peptides in DPC/LPAP detergent suspensions relative to the values observed in aqueous solutions are summarized in Table III. The changes measured for the resonances of p-MBP in DPC/LPAP mixtures were similar but smaller in magnitude than those observed for the same protein in DPC solutions (Mendz et al., 1984). Most of the chemical shift changes observed in the spectra of the peptides were comparable to those observed in the spectra of the proteins. Notable exceptions were the changes in the spectral

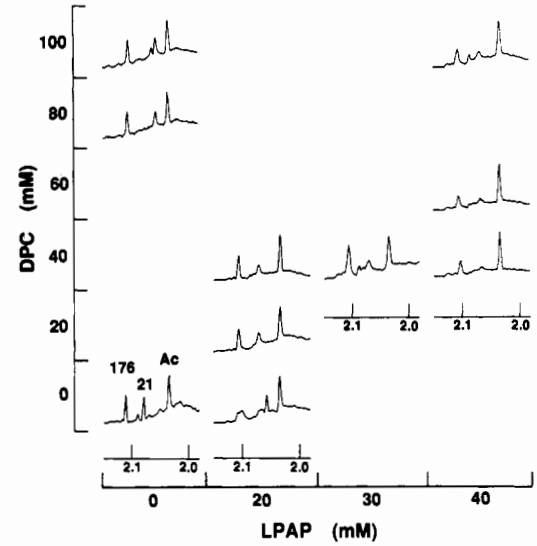

FIGURE 4: Aliphatic region of the ^{1}H NMR spectrum of porcine MBP (1.0 mM) in mixed DPC/LPAP detergent solutions at 298 K, pH 2.9. M-176 and M-21 indicate the ϵ-methyl resonances of the methionyl residues, and Ac the methyl resonance of the *N*-acetyl moiety at the beginning of the protein sequence. The concentration of detergents in each mixture is indicated in the scales.

position of the resonances corresponding to Y-128, Y-135, and H-139; the values measured in the spectrum of peptide 99–179 or r-MBP were different from those in the spectrum of p-MBP (Table III). Since the peptide was derived from the rabbit protein, an explanation for these differences could be the significant variations found in this region of the primary structure of MBP from rabbit and pig. In the segment comprising residues 120–140, A-133 and S-137 in r-MBP are substituted by proline residues in p-MBP. Variations in the conformation of this region of MBP from different species were established in NMR studies of the interactions of the protein with a monoclonal antibody to an epitope consisting of the linear sequence of residues 130–139 of bovine MBP (Mendz et al., 1985). The fact that some resonances of peptide 99–179

Table IV: Calculated Average Hydrophobicity of r-MBP Peptides[a]

peptide	average hydrophobicity	% of cationic residues	segments of possible amphipathic helices
1–44	0.175	20	13–28: K⁺(Y)LATASTMD⁻(H)AR⁺HGF 35–44: (T)GILD⁻SIGR⁺(F)
45–90	0.333	25	58–73: K⁺D⁻HA(A)R⁺TTHYGSL(P) 87–90: VVHF
1–98	0.225	20	13–28: K⁺(Y)LATASTMD⁻(H)AR⁺HGF 35–45: (T)GILD⁻SIGR⁺(F)F 58–73: K⁺D⁻HA(A)R⁺TTHYGSL(P) 87–97: V(V)HFFK⁺NIVT(P)
91–179	0.221	25	91–97: FK⁺NIVTP 108–119: R⁺(G)TVLSR⁺FSW(G)A 140–159: K⁺GLK⁺G(A)D⁻AQGTLSR⁺(L)F
99–179	0.254	25	108–119: R⁺(G)TVLSR⁺FSW(G)A 140–159: K⁺GLK⁺G(A)D⁻AQGTLSR⁺(L)F

[a] Segments predicted to be capable of forming amphipathic helices are shown. Positive values indicate that the peptide is, on the whole, hydrophilic. Underlined and nonunderlined residues project from different halves of the helical wheel, with underlined residues on the hydrophobic side and circled residues at the boundary. Acidic and basic residues are indicated with negative and positive signs, respectively. The proline residues in segments 58–73 and 87–97 are not considered to be in the helices; their orientations in the helical wheel are shown for completeness.

and r-MBP were broadened in mixed detergent suspensions and the corresponding resonances in the spectrum of p-MBP were not suggested a tighter binding to the micelles and/or the formation of large peptide/protein detergent aggregates in the former case.

When a protein/detergent mixture at a molar ratio 1:200 was lyophilized from H_2O and then suspended in ^2H_2O for NMR measurements, all exchangeable hydrogens were undetectable after 30 min. Thus the binding of DPC/LPAP micelles did not protect any peptide or amide hydrogens from rapid exchange with the surrounding water.

Figure 5 shows the resonances corresponding to the *N*-Ac-CH_3 and M-ϵ-CH_3 moieties of the protein spectrum in mixed detergent solutions (1:150) to which spin-labeled stearic acids were added. The concentration of protein was 1.0 mM and that of spin-labels 0.4 mM. Electron paramagnetic resonance and ^{13}C NMR studies (Mendz et al., 1988) have shown that these spin-labels are incorporated into DPC micelles and DPC/MBP complexes. For the MBP/DPC/LPAP complexes, addition of small amounts of spin-label did not change the chemical shifts of assigned resonances. This result indicated that, at least at the concentrations employed, the stearic acids did not perturb the conformational characteristics of the protein/detergent complexes. The resonances of various methyl groups showed different effects due to the spin-labels. The *N*-Ac-CH_3 resonance was not affected by any of the labels, whereas that corresponding to M-176 was broadened by all the labels. An interesting observation was that the downfield resonance of M-21-ϵ-CH_3 was not affected by the C_5 label and was broadened by the C_{12} and the C_{16} labels, whereas the upfield resonance was broadened by all three labels. These results suggested that the split M-21-ϵ-CH_3 resonances arise from residues in protein molecules with two conformations in which M-21 is located at different depths in the detergent micelles.

The average hydrophobicity of rabbit MBP peptides calculated by the method of Hopp and Woods is given in Table IV. An analysis of the r-MBP sequence by helical wheels (Schiffer & Edmundson, 1967; Randall & Zand, 1985) and space-filling models for possible amphipathic helices yielded the segments listed in Table IV. Underlined residues would be located on the hydrophobic side of the helix and nonunderlined residues on the hydrophilic side, with circled residues at the boundary.

Model building was required in the analysis to take into account the flexibility of basic residue side chains and the orientation of aromatic rings. The segments in an α-helical

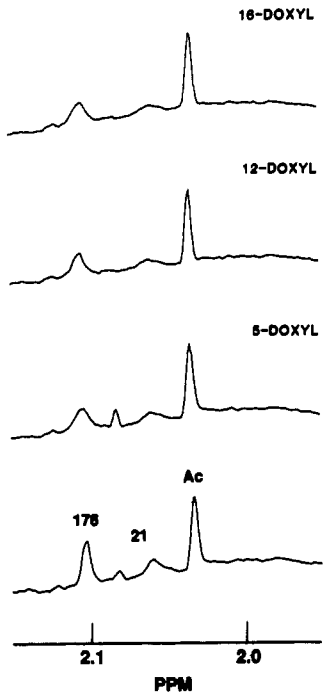

FIGURE 5: Effects of incorporation into DPC/LPAP micelles of spin-labeled fatty acids on the methionyl ϵ-CH_3 and *N*-Ac-CH_3 resonances of the ^1H NMR spectra of porcine MBP in the detergent solutions at 298 K, pH 2.9. The MBP/DPC/LPAP/spin-label molar ratio was 1:120:30:0.4.

conformation could interact hydrophobically with the micelles. The underlined and nonunderlined residues in the sequences given in Table IV project from different halves of the helical wheel; the circled residues would lie at the boundary. Acidic and basic residues in the sequences have been indicated with negative and positive signs, respectively. The segments begin and generally end with residues capable of penetrating into the micelle core. Basic residues are permitted at the beginning of the sequence because their side chains can lie back toward the N-terminus, with the cationic group located at the micelle/solvent interface or interacting with the head groups of the anionic detergents. In a survey of 43 protein structures Gray and Matthews (1984) found that approximately 70% of the serine residues and 85% of the threonine residues located in helical segments make hydrogen bonds to carbonyl oxygen atoms in the preceding turn of the helix. This bonding within a helix provides a way for these residues to satisfy their hydrogen-bonding potential, permitting them to occur in helices

buried within a hydrophobic milieu. It is then valid to assign serine and threonine residues to the hydrophobic face of the possible amphipathic helices given in Table IV. Each helix can be rotated slightly about its axis to optimize its location in the micelle interior while still maintaining an outward orientation for the charged residues.

Comparison of the number of residues included in helices in each of the r-MBP peptides (Table II) with the number of residues in the possible amphipathic helices (Table IV) showed that not all the potential helices would be realized in the interactions with DPC/LPAP micelles. In peptide 1–44 only part of one of the segments would be in a helical conformation. The 13 helical residues of peptide 45–90 could be located in segment 58–73. The 16 residues in helices in peptide 1–90 could be located in one of the two long segments 13–28 or 58–73, or, alternatively, they may form shorter helices in these segments, or in one of them and in segment 35–44. At least three of the segments in peptide 1–98 would adopt partially helical conformations; if the helices were confined to the sequences limited by residues at the micelle/solvent boundary, there would be an amphipathic helix in each of the four segments. α-Helices in both segments 108–119 and 140–159 of peptide 99–179 would be required to account for the 30 residues in helical conformation; but the 11 residues in helices in peptide 91–179 would require only one of the segments.

Discussion

The CD and NMR data on the changes observed in the protein spectra (Table I, Figures 2 and 3) demonstrated that conformational changes occur as a result of the interaction of MBP with anionic and mixed neutral/anionic detergent micelles. The data suggested that there are discrete interaction sites in the protein molecule for neutral and for anionic detergents, that at least some of these sites are different for each type of detergent, and that the protein adopts conformations which are partially different in the interactions with neutral, anionic, or mixed detergent micelles. Binding of MBP also produced different macroscopic effects in the detergent suspensions. Formation of aggregates detected by an increase in turbidity have been reported in protein suspensions with charged detergents (Jones & Rumsby, 1978; Smith & McDonald, 1979). Insoluble aggregates were observed in MBP suspensions with LPAP, LPS, and SDS, but not with DPC. These effects can be explained by assuming that the forces binding MBP to neutral detergent micelles would be predominantly hydrophobic and would require the arrangement of specific protein segments into the micelle core with each micelle not attached to more than one protein molecule, whereas the prevalent forces in the interactions with negatively charged detergents would be ionic, at least at high detergent/protein molar ratios, and would require only nonspecific close proximity between cationic groups in the MBP molecule and anionic groups in the detergent, allowing the attachment of more than one protein molecule to a single micelle in the cases in which the detergent was in micellar form. The formation of aggregates in MBP/anionic detergent suspensions could have also been affected by the shape of the micelles. Ionic detergents tend to form rod-shaped micelles at high concentrations (Lindman & Wennerström, 1980). Mazer et al. (1976) investigated the micellar phase of SDS in aqueous sodium chloride solutions using quasi-elastic light scattering spectroscopy. They found that, at room temperature, low electrolyte concentration ($\leq$0.15 M), and up to an SDS concentration 8 times the critical micelle concentration, the hydrodynamic radius of the micelles was 2.5 nm, in satisfactory agreement with the values for the apparent radii of spherical SDS micelles from small-angle X-ray scattering studies. The maximum detergent concentration employed in CD measurements of SDS/MBP suspensions was 0.32 mM, an order of magnitude below 8.3 mM, the critical micelle concentration of SDS (Lindman & Wennerström, 1980). The maximum detergent concentration used in CD measurements of LPAP/MBP suspensions was 0.6 mM, between 2 and 5 times the critical micelle concentration of LPAP (Lindman & Wennerström, 1980). It is then reasonable to conclude that micelle shape did not make a significant contribution to the α-helix induced in MBP in suspensions of SDS or LPAP alone.

Interactions of MBP with mixed detergent micelles induced in the protein mole percent α-helicities that in each case had a value intermediate between those measured for suspensions of the individual component detergents (Table I). Approximately 18% helicity was induced in the protein molecules interacting with DPC micelles (Mendz et al., 1984); interaction with LPAP micelles induced conformations with approximately 40% α-helix. Binding to mixed DPC/LPAP micelles at ratios of 9:1 to 3:1 resulted in MBP conformations with helical contents between 24% and 30% at low detergent/protein ratios, and the amount of α-helix adopted by the protein depended on the molar ratio of detergents (Figure 1). At high detergent/protein ratios the induced α-helicity was approximately 20% and was independent of the detergent mixture for DPC/LPAP molar ratios between 3:1 and 9:1 (Figure 1). The changes in chemical shift and the broadening observed for specific resonances in the ^{1}H NMR spectra of MBP showed differences in the presence of DPC or the anionic detergents (Figures 2 and 3), suggesting that there are different interaction sites for each type of detergent, in agreement with the CD results. The spectra measured in detergent mixtures at high detergent/protein ratios showed changes characteristic of both detergents, with predominant features closer to those induced by the presence of the neutral detergent (Figure 4, spectrum at DPC/LPAP/MBP molar ratio 100:40:1). These effects can be followed in the spectra of the protein suspensions titrated with DPC/LPAP mixtures at various molar ratios (Figure 4). It has been reported that in the presence of amphiphilic molecules both ionic and hydrophobic interactions are important in the conformational changes observed in MBP (Anthony & Moscarello, 1971) and that at very high SDS/MBP molar ratios the molecules within the complexes rearrange to maximize hydrophobic interactions (Jones & Rumsby, 1975, 1978; Liebes et al., 1976). The data on DPC/LPAP/MBP complexes suggested that at a given detergent mixture a balance is reached between the forces inducing different conformations and that at high detergent/protein ratios the conformation adopted by the protein appeared to be closer to that induced by the neutral detergent. This interpretation also explains the conformational changes of MBP observed by CD in titrations of protein solutions with DPC/LPAP mixtures. Of special interest was the observation that at high detergent/protein ratios the net effect appeared to favor hydrophobic interactions. The observations that aggregates formed in MBP/LPAP suspensions at a molar ratio of 1:20 and LPAP concentrations above 20 mM, but that the concentration of LPAP could be raised to 40 mM in MBP/DPC/LPAP suspensions at a molar ratio of 1:40:40 (Figure 4) without any macroscopic effects of aggregation becoming apparent, supported the hypothesis that at these detergent concentrations hydrophobic interactions with specific protein segments make a greater contribution than electrostatic interactions with basic residues to the binding of the protein to the micelles.

This conclusion was also supported by the line-broadening effects observed in the ^1H NMR spectra of MBP/DPC/LPAP spin-label complexes. Spin-labels may provide an indication of the depth of penetration of certain regions of the protein into the detergent micelle. The C_5 label has been considered to be predominantly at the interface of the micelle with the external medium, the C_{12} moderately within the hydrocarbon region, and the C_{16} well within the interior of the micelle. These locales are only approximate descriptions because of the mobility of the label in and out of the micelle (Gruen, 1981; Menger & Doll, 1984). At high detergent/protein ratios broadening by the C_{12} and C_{16} spin-labels occurred in several of the observable resonances from the protein spectrum (Figure 5), indicating that segments of the protein including M-21, H-89, and M-176 were involved in hydrophobic interactions with the detergent micelles.

Cleavage of MBP at residue F-90, in the most hydrophobic region of the protein, resulted in two peptide fragments, 1–90 and 91–179, of similar size. In DPC suspensions the total number of residues in helical conformations was 31 for the intact protein and 22 for the sequential peptides 1–90 and 91–179 (Table II). The smaller number of residues in helices in the peptides may be approximately accounted for by the 10 residues that could form an amphipathic helix in segment 87–97 (Table IV) in the whole protein, but not in the peptides, where the segment is divided by the cleavage. In contrast, the number of residues in helical conformations in DPC suspensions of peptides 1–98 and 99–179, resulting from cleavage of MBP at R-98, increased to 39 (Table II). The data indicated the importance of the integrity of segment 91–98 for the formation of a helix in neutral detergent suspensions of the protein and peptides (Mendz et al., 1984). An explanation of the increase in the total number of residues in helices observed for the sequential peptides 1–98 and 99–179 relative to the intact protein could be that intramolecular long-range interactions arising from tertiary structures in MBP limit the number of protein regions that could form helices. Cleaving the protein at R-98 would have the effect of removing long-range interactions and consequently increasing the total number of helical residues in DPC suspensions of the resulting sequential peptides. It is also possible that the smaller number of residues in helices observed for intact MBP in DPC suspensions arose from constraints posed by detergent–protein interactions which would not be present in smaller fragments. In this case it is reasonable to expect that cleaving at F-90 would also procude a relaxation of those constraints. However, the result of this cleavage was a decrease in the total number of helices in the sequential peptides 1–90 and 91–179 relative to whole MBP, and the deficit could be ascribed to the loss of a helical segment in the region 87–97. The analysis suggested that in DPC suspensions the protein adopts conformations in which the number of residues in helices is limited by long-range intramolecular interactions which depended on segment 91–98 remaining an integral part of the C-terminal half of the polypeptide chain.

The α-helix content of the six r-MBP peptides increased in DPC/LPAP suspensions relative to DPC suspensions (Table II). In contrast to the 10% increase (3 residues) in helicity measured for the whole protein in mixed detergent suspensions, the increase observed for peptides 1–44 and 1–98 was approximately 30% (2 and 9 residues), and for peptides 45–90 and 99–179 the increases were 130% and 160% (7 and 19 residues), respectively (Table II). These increases could be explained by assuming that the conformational changes of the peptides in the presence of mixed detergent micelles resulted from the "added" contributions of hydrophobic interactions with DPC and ionic interactions with LPAP. This interpretation, however, would not account for the large differences between the conformational changes observed in peptides of similar size like 1–44 and 45–90, or 1–98 and 99–179. First, a comparison of the amino acid composition of the peptides showed that there is only a 5% difference between the percent of cationic residues in peptides 1–44 and 45–90 and between the percent of cationic residues in peptides 1–98 and 99–179 (Table IV). The difference in α-helical content between similar peptides did not appear to arise from their overall hydrophobicity either, because peptide 45–90 has an average hydrophilicity approximately twice that of peptide 1–44, but peptides 1–98 and 99–179 have similar average hydrophilicity (Table IV). Finally, ionic effects cannot be simply "added" to hydrophobic effects because although the α-helix of peptide 45–90 includes two residues more than the that of peptide 1–44 in DPC suspensions, the situation is reversed for peptides 1–98 and 99–179 which have 28 and 11 residues in helical conformations, respectively (Table II). There is a notable difference between the number of segments capable of forming amphipathic helices in peptides 1–98 and 99–179: four for the N-terminus peptide and two for the C-terminus peptide (Table IV). Instead of assuming the "addition" of hydrophobic and ionic effects, it is possible to consider an "accommodation" of the peptide chain in the micelles forming helices which would allow both types of interactions to take place. The spatial requirements needed to accommodate several helical segments in the detergent micelles would impose limitations on the location of cationic side chains and their possible interactions with the micellar interface. In general, the larger the number of segments that needed to be accommodated, the greater were the limitations on the locales allowed for positively charged side chains in the proximity of the micelles. The intramolecular interactions that limited the formation of helices in DPC suspensions of the intact protein could also be operative in suspensions of MBP in mixed DPC/LPAP micelles and explain why the increase in the number of residues in helical conformations in the whole protein is much smaller than the total number measured for peptides 1–98 and 99–179 (Table II). The difference observed in the number of α-helical residues in peptides 91–179 and 99–179 in DPC/LPAP suspensions (Table II) could also be attributed to intramolecular interactions present in peptide 91–179, which preserves the integrity of segment 91–98, and absent in peptide 99–179, from which the segment 91–98 has been removed. There was good agreement in the changes of chemical shifts observed for the resonances of residues 14, 21, 23, 26, 61, 69, 89, and 117, located within the potentially helical segments, when measured for r-MBP or for the derived peptides in DPC/LPAP suspensions relative to aqueous solutions (Table III). The only discrepancy in the values of the changes measured for residues in helical segments was found for the His-ϵ_1-68 resonance. These data were interpreted as suggesting that the residues were in helical conformations.

Burns and Campagnoni (1982) reported that the sum of the SDS bound to two bovine MBP cleavage fragments (peptides 1–117 and 118–179) was almost twice that bound by the intact protein at saturation. They suggested that cleavage of MBP exposed sites for additional binding of SDS that are masked either by a structural region within the intact protein molecule or by a conformational change induced in the protein by the interactions with SDS. The CD results for the DPC/LPAP complexes with MBP and peptides 1–98 and 99–179 showed a 2-fold increase in the total number of residues in helices in

both peptides relative to the protein (Table II). These data could be interpreted in terms of additional interaction sites for DPC/LPAP micelles being exposed as the result of cleaving the protein. The assumption of a higher ordered structure of MBP in aqueous solution to explain the unmasking of potential interaction sites is at variance with the spectroscopic evidence that in aqueous solutions the protein is a flexible coil devoid of α-helical conformations but that it contains a small amount of β-structure and turns (Mendz et al., 1982, 1983a,b; Mendz & Moore, 1983; Martenson et al., 1985; Stone et al., 1985; Surewicz et al., 1987; Gow & Smith, 1989). The proposal that cleavage of the protein may expose interaction sites masked by a structure induced in the whole protein by interaction with SDS detergent micelles is compatible with the interpretation that intramolecular interactions limit the formation of helices in intact MBP in DPC/LPAP suspensions. This assumption, like the hypothesis that accommodating helical segments impose restrictions on the possible ionic interactions between micelles and peptides, would involve a rearrangement of the peptide chain. These explanations are in agreement with other results reporting the rearrangement of complexes of MBP with anionic lipids to maximize hydrophobic interactions (Jones & Rumsby, 1978; Boggs et al., 1982; Surewicz et al., 1987).

It should be emphasized that the hypothesis of balanced hydrophobic and ionic effects refers to the composition of the detergent mixture and the conformation of the protein and peptides, and not to the fact that the interactions occur with detergent micelles. There is a large geometric factor that has to be taken into consideration when comparing the binding of MBP to micelles as opposed to a bilayer. The presence of micellar structures may pose particular constraints to the accommodation of hydrophobic and ionic interactions between protein and micelles that may not be encountered in binding to bilayers. But if the interactions occurred with several mixed detergent micelles, as was the case for MBP/DPC complexes (Mendz et al., 1988), it would not necessarily follow that a micellar structure would impose more restrictions on the protein conformation than those imposed by lipid bilayers. On the other hand, the surface pressure at a nearly flat bilayer can be expected to be higher than at micelles, which are more readily distortable on account of their curvature; hence it is reasonable to surmise that the protein could have a greater degree of penetration into micelles than into a bilayer. Considering that the ratio neutral/negatively charged lipids in the myelin membrane is about 4:1, the results of this study provide a stimulus for further studies on the interaction of MBP with model mixed detergent bilayers.

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