Interaction of Myelin Basic Protein with Micelles of Dodecylphosphocholine†

George L. Mendz,* Walter J. Moore, Larry R. Brown, and Russell E. Martenson

ABSTRACT: Interactions of myelin basic protein (MBP) and peptides derived from it with micelles of dodecylphosphocholine (DPC) and perdeuterated DPC have been studied by proton nuclear magnetic resonance (NMR) at 400 MHz and by circular dichroism (CD). When MBP binds to DPC micelles, it acquires about 18% α-helicity. The CD spectra of various peptides derived by cleavage of MBP indicate that a major α-helical region occurs in residues 85–99 just before the sequence of three prolyl residues 100–102. From line broadenings by fatty acid spin-labels in the micelles and from changes in chemical shifts, the NMR data identify specific residues in MBP that participate in lipid binding. One such sequence is an α-helical region from residues 85 to 95, and others occur around methionine-21 and between residues 117 and 135. The different effects of C_5, C_{12}, and C_{16} spin-labels suggest that some segments of the protein may penetrate beyond the dipolar interfacial region of the micelles into the hydrophobic interior, but no part of the protein is protected by the micelles against rapid exchange of its amide groups with the aqueous environment. Even at a lipid to protein molar ratio of 200/1, most NMR resonances from side chains of amino acid residues are not appreciably broadened, suggesting that much of the polypeptide remains highly mobile.

The role of myelin basic protein (MBP) in the formation, maintenance, and breakdown of myelin membranes in the mammalian central nervous system has been the focus of much experimental research [for reviews, see Carnegie & Moore (1980) and Boggs et al. (1982)]. High-resolution nuclear magnetic resonance (NMR) is potentially one of the most effective ways to investigate interactions of MBP with membrane lipids. Previous work has considered effects of MBP on the NMR spectra of lipids (Deber & Young, 1979; Smith et al., 1983) and some aspects of the protein spectrum in the presence of lipids (Littlemore & Ledeen, 1979; Smith, 1982; Deber et al., 1978). The NMR spectra of proteins bound to lipid bilayers or vesicles are usually so broadened that the methods of high-resolution NMR are not applicable. Therefore, it has been necessary to restrict the lipid components in the protein–lipid systems to those that form micelles. Spectra of small proteins bound to lipid micelles are often sharp and well resolved so that it is possible to follow changes in the resonances of specific amino acid residues in the protein–lipid system. The use of perdeuterated lipid permits recording of the protein spectra with minimal interference from resonances due to the lipid (Brown, 1979; Feigenson & Meers, 1980).

The possibility of such a study depends on the exact assignment of resonances in the protein. The technique is to assign these resonances in aqueous solution and then to follow them as increasing amounts of lipid are added to the protein solution. This paper is based on previous NMR studies of MBP in which assignments of threonine, tyrosine, histidine, and other residues have been made (Mendz et al., 1982, 1983a,b; G. L. Mendz, W. J. Moore, and R. E. Martenson, unpublished results). Fatty acid spin-labels incorporated into the micelles provide an indication through broadening of NMR resonances of certain amino acid residues that lie in or on protein-bound micelles (Brown et al., 1981).

† From the School of Chemistry, University of Sydney, N.S.W. 2006, Australia (G.L.M. and W.J.M.), the Michigan Molecular Institute, Midland, Michigan 48640 (L.R.B.), and the National Institute of Mental Health, Bethesda, Maryland 20205 (R.E.M.). *Received April 9, 1984.* This work has been supported by the National Health and Medical Research Council and by Esso Australia Ltd. L.R.B. acknowledges financial support from the National Institutes of Health (Grant GM30556).

The lipids used are dodecylphosphocholine (DPC) and [$^2H_{38}$]dodecylphosphocholine ([$^2H_{38}$]DPC). [$^2H_{38}$]DPC has a molecular mass of 389 daltons and forms micelles in aqueous suspensions with a critical micelle concentration (cmc) of about 1.0×10^{-3} M (Lauterwein et al., 1979) at 295 K, with 56 ± 5 molecules per micelle. Thus, the mass of a micelle in the absence of protein is about 21 800 daltons, compared to 18 500 daltons for the protein.

Myelin basic protein in aqueous solution has little if any (<4%) α-helical content, but on interaction with various lipids, it acquires varying fractions of α-helicity (Keniry & Smith, 1981). In this paper, circular dichroic (CD) spectra are used to estimate the α-helicity in MBP and peptides derived from it when they interact with DPC micelles. In this way, regions of the polypeptide chain that form α-helix in the presence of DPC are localized, and such information can be correlated with the corresponding NMR spectra.

Materials and Methods

Porcine MBP (p-MBP) was kindly provided by Dr. Max Marsh of Eli Lilly & Co. Peptides were prepared from rabbit MBP (r-MBP) by cleavage with porcine pepsin (EC 3.4.23.1) or human thrombin (EC 3.4.21.5) and purified as previously described (Martenson et al., 1981a,b; Law et al., 1984). 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. DPC and [$^2H_{38}$]DPC were synthesized by methods described (Brown, 1979; Lauterwein et al., 1979).

The proteins were lyophilized twice from 99.5% D_2O and dissolved in 99.96% D_2O (Merck Sharp & Dohme) at a concentration of 0.50–1.0 mM in 5-mm OD precision NMR tubes. The pH was adjusted with DCl or NaOD and measured with an Ingold 6030-02 microelectrode, values being reported as meter readings. Increasing amounts of DPC were added to the protein solutions to obtain a series of DPC/MBP molar ratios from 0 to 500.

The NMR measurements were made with a Bruker WM 400 spectrometer. Spectra were taken at 313 K by accumulating 1000–2000 transients over 16K data points. A radio-frequency pulse of 90° (7–9 μs) was used with a spectral width of 5000 Hz and a repetition time of 1.90 s. Chemical shifts were measured relative to internal DSS (sodium 4,4-di-

0006-2960/84/0423-6041$01.50/0

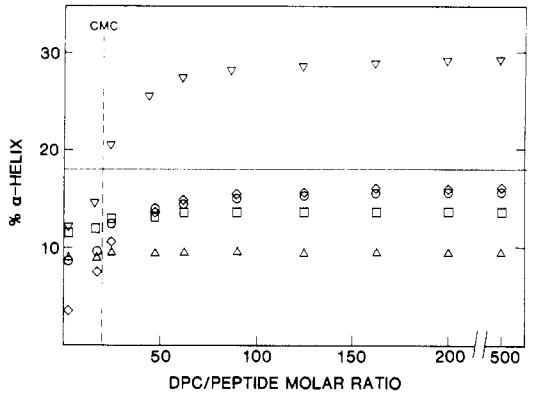

FIGURE 1: Calculated percent α-helicities of myelin basic protein and derived peptides as functions of lipid/protein molar ratios at pH 6.5. The standard numbering of the amino acid sequence is shown in Figure 8: (△) 1–44; (□) 45–90; (○) 1–90; (▽) 1–99; (◇) 100–179. The horizontal line denotes the maximum value for MBP.

methyl-4-silapentane-1-sulfonate) by the method previously described (Mendz et al., 1982). The exchange with D_2O of –NH and $-NH_2$ groups in the protein–lipid system was followed by lyophilizing a sample of MBP with [$^2H_{38}$]DPC from H_2O, suspending the mixture in D_2O, and taking 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 DPC–MBP aqueous suspension to obtain the final composition desired, lyophilizing the mixture, and dissolving it again in D_2O.

CD spectra were measured at 298 K with a JEOL-500C spectropolarimeter at various lipid/protein ratios. Samples were prepared in quartz cells with path lengths from 0.1 to 5.0 mm. Concentrations of peptides were usually 0.06 mM and of proteins, 0.008 mM. Measurements were made at pH 6.5, but there was no change with pH from 3.7 to 6.8. The CD of lipid alone was negligible over the spectral range recorded.

Results

Circular Dichroism. A convenient approximate measure of α-helicity is the molar ellipticity at 222 nm, $[\theta]_{222}$. The values of $[\theta]_{222}$ as a function of α-helicity were determined by fitting the entire CD spectrum to a weighted average of α-helical, random-coil, and β-sheet spectra by the method of Keniry (1981). Absolute values of α-helicity are uncertain to about ±4%, but comparative values are reproducible to ±0.5%.

The CD spectra of p-MBP and a number of peptides derived from r-MBP were measured in aqueous solution with increasing concentrations of DPC up to a DPC/MBP molar ratio = 500. Figure 1 shows the values of $[\theta]_{222}$ and the calculated percent α-helicity of the protein and peptides as functions of [DPC]/[MBP]. The α-helicity of the various peptides in aqueous solution and the maximal α-helicity reached in the lipid titrations are summarized in Table I.

Figure 2 shows $[\theta]_{222}$ for three concentrations of MBP with increasing molar ratios of DPC/MBP. The critical micelle concentrations (cmc) in Figures 1 and 2 are values for DPC in the absence of protein. At the lowest concentrations of MBP, changes in the CD spectra are complete at DPC concentrations less than twice the cmc of DPC (□, Figure 2). At higher MBP concentrations, changes in the CD spectrum are complete only when the DPC/MBP molar ratio is about 200, indicating that each MBP molecule binds at least 200 DPC molecules. These results ensure that under the conditions used for the NMR experiments described below, essentially all MBP

Table I: Mole Percent of α-Helix at 295 K of MBP and Its Peptide Fragments in Water and in the Presence of DPC Micelles

peptide	mol % of α-helix	
	H_2O	DPC
1–44	8.9	9.5
45–90	11.5	13.5
1–90	8.6	15.8
1–99	11.8	28.8
100–179	4.4	16.0
1–179	4.0	18.5

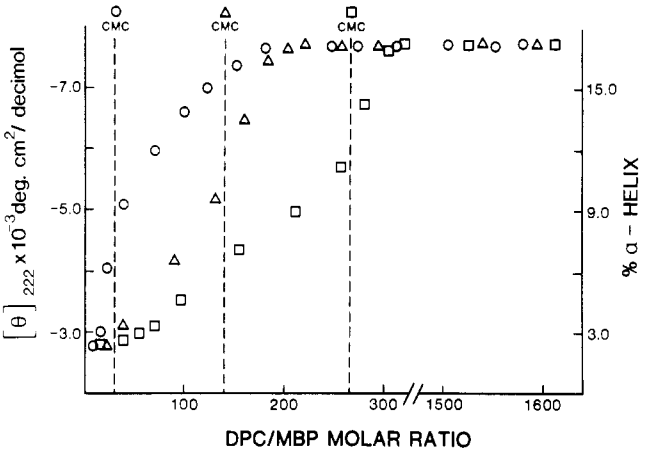

FIGURE 2: $[\theta]_{222}$ and α-helicity of myelin basic protein as functions of the lipid/protein molar ratio. Protein concentrations: (○) 0.035 mM; (△) 0.008 mM; (□) 0.004 mM.

is bound to micelles and each MBP molecule has a full complement of bound lipid. The final value of $[\theta]_{222}$ is independent of MBP concentraton and is reached at DPC/MBP molar ratios of 200 even for a 5-fold change in MBP concentration (○, △, Figure 2). Therefore, under the conditions of the present experiments, aggregation of MBP does not appear to occur in the presence of excess DPC.

Proton Magnetic Resonance. Myelin basic protein aggregates even at quite low concentrations at pH 6 in aqueous solutions (Chapman et al., 1978). Therefore, the NMR spectra reported here have been obtained at low concentrations and low pH. This restriction to low concentrations of protein has prevented the application of the powerful methods of two-dimensional (2D) NMR that have given such excellent data on details of protein–lipid interactions in more amenable systems (Braun et al., 1983).

The bottom portion of Figure 3 shows the ^1H NMR spectrum of p-MBP (0.50 mM) at pH 3.70 in D_2O solution at 313 K, and the top portion of Figure 3 is the spectrum under the same conditions except for addition of [$^2H_{38}$]DPC at a lipid/protein molar ratio of 200. The use of perdeuterated lipid allows almost all the protein resonances to be observed without interference from those of the lipid. At the concentrations used, resonances from residual ^1H in the lipid are usually of low intensity; however, such resonances from the lipid CH_2 groups appear in a large broad peak at about 1.25 ppm. The protein spectra remain generally sharp and well resolved in the presence of the lipid. There is some broadening of resonances compared to unbound MBP, but considering that a complex containing 1 MBP and 200 DPC molecules corresponds to a particle mass of 96 300 daltons, of which MBP = 18 500 daltons, the NMR spectrum shows surprisingly sharp protein resonances.

Although most of the resonances from the protein are not greatly broadened, some exceptions are noted. The peaks in the region around 4.0 ppm, which include the α-CH resonances of the polypeptide main chain, are considerably broadened by

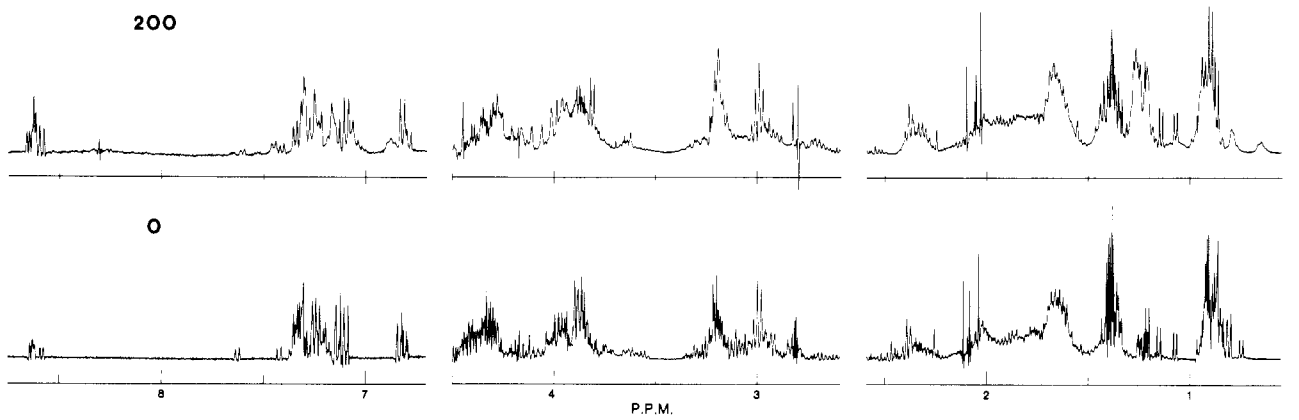

FIGURE 3: (Bottom spectrum) Proton NMR spectrum of porcine myelin basic protein (0.50 mM) in D_2O at 313 K, pH 3.70. (Top spectrum) Same as bottom spectrum except for addition of [$^2H_{38}$]dodecylphosphocholine at [lipid]/[protein] = 200.

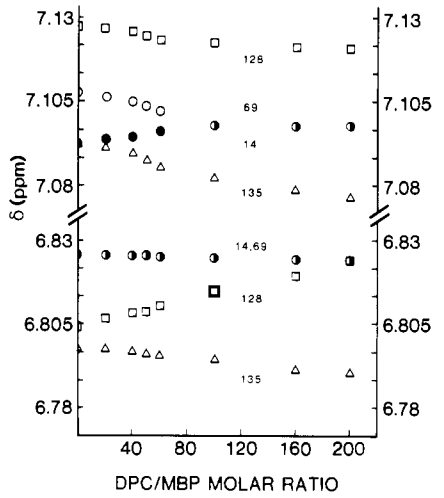

FIGURE 4: Variation of chemical shifts of δ- and ε-CH resonances of tyrosine residues at 313 K of myelin basic protein: dependence on lipid/protein molar ratio.

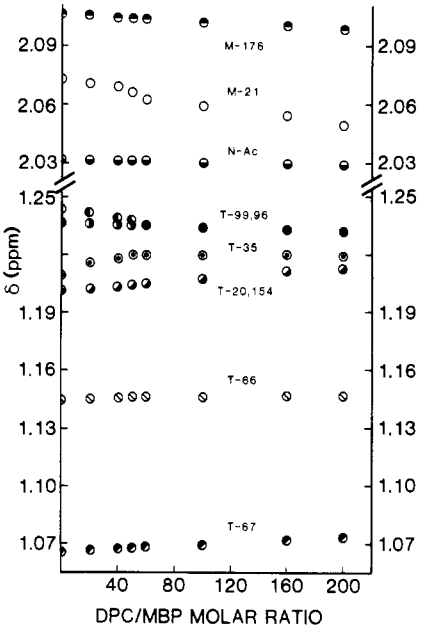

FIGURE 5: Variation of chemical shifts of side-chain methyl resonances of myelin basic protein: dependence on lipid/protein molar ratio.

the lipid. This result suggests that the effects of lipid on the secondary structure of the protein, shown by the CD data, result in broadening of some resonances from sites in or near the polypeptide backbone. On the other hand, aromatic and aliphatic side chains are usually not appreciably broadened by interaction of the protein with the DPC micelles.

Bovine and porcine MBP both contain four tyrosine residues, Y-14 -69, -128, and -135. Figure 4 shows the variation with lipid/protein ratio of the chemical shifts of the δ-CH tyrosine resonances around 7.0 ppm and the ε-CH resonances around 6.8 ppm. The largest change in chemical shift occurs for the Y-128 ε-CH resonances. Some of the tyrosine side-chain resonances are scarcely changed at all in the presence of the lipid, the Y-14 ε-CH and Y-69 ε-CH resonances remaining practically constant. The Y-69 δ-CH resonance, however, displays an appreciable upfield shift. Most of the resonances reach practically constant chemical shifts at the highest lipid/protein ratio of 200, but the Y-135 δ,ε-CH's are still moving upfield, and the Y-128 ε-CH is still moving downfield.

Figure 5 shows the effects of increasing lipid/protein ratio on some of the resonances assigned to side-chain methyl groups in MBP. Of the two methionine methyls, that of M-21 is more affected than that of M-176. These two residues serve as convenient markers for the N- and C-terminal regions of the protein, respectively. The resonance of the methyl of the N-terminal acetyl group is not changed at all by addition of lipid. Changes in the threonine methyl resonances are small, but significant effects are observed for T-20, -35, -99, and -154. The changes are practically complete by the time [DPC]/[MBP] = 50 is reached, at which point there may be about one micelle for each protein molecule. In contrast with this behavior, the chemical shift of the M-21 CH_3 is still increasing at the highest lipid/protein ratio.

Effects of lipid on chemical shifts considerably larger than those so far described are observed for the histidine ϵ_1-CH resonances summarized in Figure 6. The most striking effect is on H-89 ϵ_1-CH, which moves markedly downfield on addition of lipid. By contrast, H-23, -61, -62, and -78 are only slightly affected, and then only at high lipid/protein ratios. H-10 and H-32 show moderately large downfield shifts, and H-68 shows a small upfield shift. The shift of H-68 is the only one that has not reached a steady value as [DPC]/[MBP] = 200 is reached. These measurements were made at pH 3.70. As the pH is raised to 7.0, the histidine ϵ_1-CH resonances become broadened and indistinguishable from one another, both in aqueous solution and in the presence of lipid. This result indicates that the histidine side chains are in a regime of rapid exchange between charged and uncharged forms, and there is no detectable stabilization of any of the uncharged forms in the presence of lipid.

When a protein-perdeuterated lipid mixture at [DPC]/[MCP] = 200 was lyophilized from H_2O and then suspended in D_2O for NMR measurements, all exchangeable hydrogens

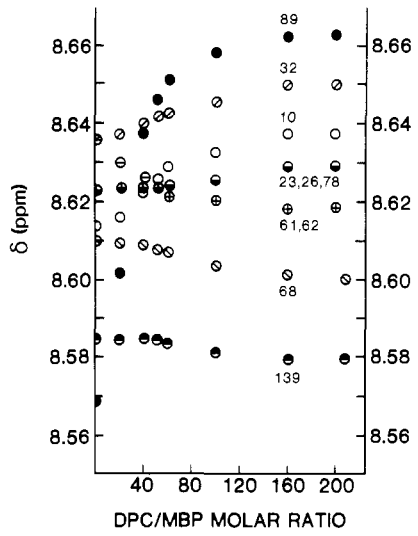

FIGURE 6: Variation of chemical shifts of ϵ_1-CH histidine resonances of myelin basic protein: dependence on lipid/protein molar ratio.

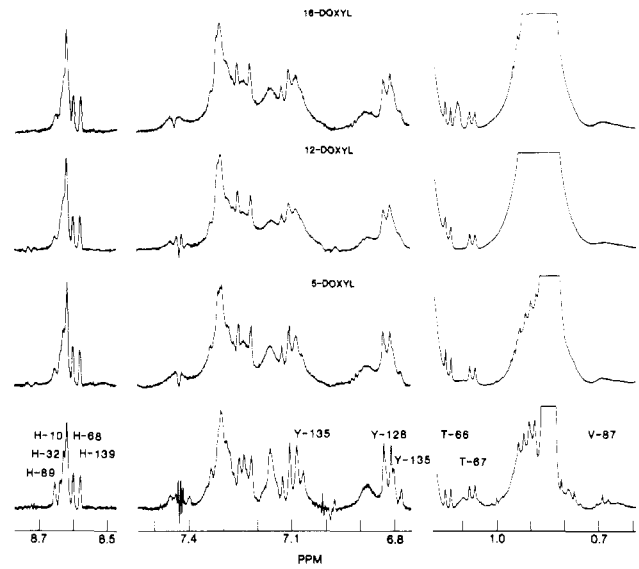

FIGURE 7: Effects of incorporation into dodecylphosphocholine micelles of spin-labeled fatty acids on the NMR spectra of myelin basic protein interacting with the lipid micelles ([DPC]/[MBP] = 60/1): (from bottom to top) (a) no spin-label; (b) 5-doxylstearate; (c) 12-doxylstearate; (d) 16-doxylstearate.

were undetectable after 30 min. Thus, the binding of DPC micelles to MBP does not protect any peptide or amide hydrogens from rapid exchange with the surrounding water.

Effects of Spin-Labels. Figure 7 shows the results of experiments in which spin-labeled stearic acids were added to the protein–lipid mixtures at a DPC/MBP molar ratio of 60. The concentration of protein is 1.0 mM and that of spin-label 0.40 mM. Electron paramagnetic resonance (EPR) and ^{13}C NMR studies by Brown et al. (1981) have shown that these spin-labels are incorporated into DPC and mixed DPC–glucagon micelles. For the MBP–DPC complexes, addition of small amounts of spin-label did not change the chemical shifts of the assigned resonances. This result indicates that, at least at the low concentrations used, the spin-labels do not perturb the conformational characteristics of the MBP–DPC complex.

Several histidine and tyrosine resonances are affected by the spin-labels. The ϵ_1-CH resonance of H-89, the chemical shift of which was seen in the lipid titration in Figure 6 to be most changed by DPC, is broadened considerably with the C_5, C_{12}, and C_{16} spin-labels, the effect of C_{12} being more marked than that of either C_5 or C_{16}. The corresponding protons of H-32 and H-10 also are broadened by the spin-labels, but the effect is not so marked as that with H-89. Among the tyrosine resonances, ϵ-CH and δ-CH of Y-135 are most affected by the spin-labels, C_{12} having a greater effect than C_5 or C_{16}. The resonances of Y-128 are also strongly affected by the spin-labels.

The resonances of various methyl groups show quite different effects due to the spin-labels. The M-176 resonance is not affected by any of the labels, whereas the M-21 is broadened and loses intensity in the presence of the C_5 and the C_{12} labels, but is hardly affected by the C_{16} label. This result is consistent with the chemical shift behavior shown in Figure 5. The resonances of upfield methyl groups, in particular V-87 or V-88, are broadened by all three doxylstearates and become unobservable. The CH_3 resonances of T-66 and T-67 are not broadened by the C_5 label and are only slightly broadened by the C_{12} label, although other methyl groups are more strongly affected. Unfortunately, the resonances of T-96 and T-99 are obscured by the methylene groups of the DPC in this experiment and are not observed.

Discussion

The CD and NMR results both demonstrate that conformational changes occur in the protein as a result of its interaction with DPC. When DPC is complexed with mellitin (Lauterwein et al., 1979) or glucagon (Bösch et al., 1980; Brown et al., 1981), the micelle size decreases from about 55 to about 40 DPC molecules. The 200 DPC molecules that bind to MBP might therefore suffice to constitute 3–5 independent lipid domains along the MBP amino acid sequence. Since NMR indicates that there are discrete lipid binding sites on the MBP molecule, a possible model may be suggested in which several micelles become attached to these sites like balls on an extended chain.

In a protein like MBP, which has an extended aperiodic structure in aqueous solution, it is reasonable to assume that the α-helicity induced in the presence of lipids is due to features in its primary structure and is not due to long-range tertiary structures (for which no experimental evidence exists). Therefore, we can use the CD data on peptide fragments of the protein to delineate the regions that become α-helical in the whole protein, although we must consider effects caused by cleavages in especially lipophilic regions of the protein. The CD data lead to the conclusion that the region of the protein on the N-terminal side of the triprolyl sequence (residues 100–102) contains most of the segments that acquire α-helicity on binding to DPC micelles. The region 1–98 acquires 28% α-helicity or an estimated 28 residues. When the protein is cleaved at the pair of phenylalanine residues at positions 90–91, eight residues are lost, and the α-helicity is decreased to 16%. Thus, it is reasonable to conclude that residues 90–91 are included in an α-helical region that can be estimated to extend from about 85 to 96, where it approaches the triprolyl sequence. In this connection, however, it is interesting to note that the 22-residue peptide comprising residues 91–112 does not itself acquire α-helicity in the presence of DPC micelles. The exact location of the remaining α-helical region(s) in the N-terminal section of the protein cannot yet be specified. The region of M-21 is not likely to be α-helical in view of the negative CD results for peptide r(1–44).

The most interesting features of the ^1H NMR spectra obtained from MBP–DPC complexes are the relatively narrow line widths, the quite small changes in chemical shifts compared to the NMR spectrum of free MBP, and the ability of

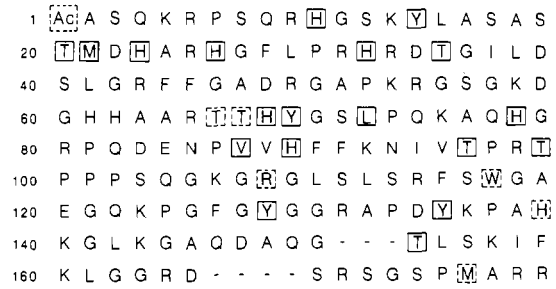

FIGURE 8: Summary of the effect of binding of dodecylphosphocholine micelles on the chemical shifts of assigned residues in porcine myelin basic protein: (solid boxes) appreciable effect found; (broken boxes) no appreciable effect found.

the spin-labels to broaden resonances from residues at very different positions in the MBP amino acid sequence. The fact that none of the protein NH and NH_2 groups are protected by the lipid against exchange with D_2O is evidence suggesting that (1) the interaction of MBP with DPC is confined to the interfacial region of the micelles or (2) the parts of the protein that penetrate the micelle move rapidly between lipid and aqueous environments.

The NMR data (chemical shifts and spin-label line broadenings) lead to the designation of specific residues that are involved in interaction with the lipid. They support the conclusions from CD, and thus the identifications of major sites of interaction of MBP and DPC micelles are well established. Figure 8 provides a summary of the effects of lipid interactions on the chemical shifts of side-chain protons in the primary structure of p-MBP [as recently reported by Kira et al. (1984)]. The interaction with lipid is not restricted to any one region of the polypeptide sequence although appreciable changes in chemical shifts are more frequent in the N-terminal portion of the chain.

In most cases, the changes in chemical shifts are not large, and H-89 displays the largest shift, +0.1 ppm. H-89 occurs in one of the most lipophilic regions of the polypeptide, between a pair of phenylalanines and a pair of valines. In the bottom spectrum of Figure 3, the most upfield doublet has been assigned to V-87 or -88 (Mendz et al., 1982). In the top spectrum of Figure 3, these peaks have broadened considerably, although most other upfield peaks have remained sharp in the presence of lipid. The effect of the spin-labels on these resonances is seen in Figure 7. These results support the conclusion from the H-89 shift: the region from V-87 to F-91 is a strong binding site for lipid.

NMR evidence shows that another lipid binding site in the N-terminal half of the protein is likely to be in the neighborhood of M-21. Previous NMR studies with other lipids have identified this locus of lipid interaction (Deber et al., 1979; Littlemore & Ledeen, 1979). The NMR results in Figure 8 are evidence for side-chain–lipid interactions of residues 20–26. The CD data lead to the conclusion that this interaction occurs without induction of α-helicity (Table I).

The sole methylated arginine in the protein at position 108 is a convenient marker for the middle region of the protein. Littlemore & Ledeen (1979) observed broadening of this resonance on interaction of the protein with micelles of lysophosphatidylcholine or ganglioside G_{M1} whereas, at lower lipid/protein ratios, Smith (1982) found no such broadening with lysomyristoylphosphatidylcholine. In the present study, the R-108 methyl peak does not undergo appreciable broadening or change in chemical shift even at [lipid]/[protein] = 200. Thus, there is no evidence that this region of the protein is involved in binding DPC micelles. The structure of a peptide that spans the triprolyl sequence at positions 100–102 suggested that the three proline residues act as a rigid barrier between a more lipophilic and a less lipophilic region of the protein chain (Nygaard et al., 1984). This conclusion is supported by the present evidence for lipid interaction in the region of residues 85–98 and the absence of interaction at residue 108.

The line-broadening effects observed in the ^1H NMR spectra of MBP–DPC spin-label complexes together with the chemical shift differences between free and lipid-bound protein allow the portions of MBP that are in contact with the lipid components to be approximately delineated. In general, the greatest broadening by the spin-labels occurs in those residues for which the chemical shifts are most affected by lipid binding.

So far, resonance assignments have been obtained for only seven residues in the C-terminal region of MBP, i.e., residues 100–179. Little or no line broadenings or chemical shift changes are observed for R-108, H-139, or M-176. Moderate broadening is observed for W-117, Y-128, and Y-135. Thus, the region including residues 117–135 is likely to be bound to lipid.

Spin-label experiments may provide an indication of the depth of penetration of certain regions of the protein into the lipid micelle. The C_5 label has been considered to be predominantly in the zwitterionic outer interface of the micelle, the C_{12} label moderately within the hydrocarbon region, and the C_{16} label well within the interior of the micelle. In fact, these locales must be qualified to take into account the mobility of the label. Theoretical calculations (Gruen, 1981) and experimental observations (Menger & Doll, 1984) have shown that the nonpolar terminal groups spend considerable time close to the interfacial regions of the micelle. The observation that C_{12} spin-labels sometimes cause more broadening than either C_5 or C_{16} may be due to this effect.

The suggested model of discrete local interactions is consistent with results of earlier studies of smaller polypeptides such as mellitin (26 residues) and glucagon (30 residues) bound to DPC micelles (Lauterwein et al., 1979; Brown et al., 1981). For example, most of the resonances from amino acid side chains of mellitin showed only small changes in chemical shift upon binding to DPC micelles, even though binding involved a change in conformation from a flexible, extended chain to a largely α-helical structure (Brown & Wüthrich, 1981; Terwillinger et al., 1982). For glucagon bound to DPC micelles, regions of the polypeptide chain that were in contact with the micelle surface but had extended conformations gave quite sharp ^1H NMR resonances (Brown et al., 1981). A full explanation of the narrow line widths observed in polypeptide–micelle complexes has not yet been advanced; however, possible explanations of why such complexes seem to yield narrower ^1H NMR line widths than usually observed for globular proteins of comparable mass would include greater side-chain mobility than is typical of the interior of a globular protein and larger hydrogen–hydrogen distances through interspersion of lipid between different portions of the polypeptide chain (Wüthrich et al., 1980).

The surprising sharpness and detail observed in the ^1H NMR spectra of micele-bound MBP and the specific assignments already available for 25 residues provide an excellent opportunity for further investigations of the interactions between MBP and lipids.

Registry No. DPC, 29557-51-5.

References

Boggs, J. M., Moscarello, M. A., & Papahadjopoulos, D. (1982) in *Lipid–Protein Interactions* (Jost, P. C., & Griffith, O. M., Eds.) pp 1–51, Wiley, New York.

Bösch, D., Brown, L. R., & Wüthrich, K. (1980) *Biochim. Biophys. Acta 603*, 298-312.
Braun, W., Bösch, C., Brown, L. R., Gō, N., & Wüthrich, K. (1981) *Biochim. Biophys. Acta 667*, 377-306.
Braun, W., Wider, G., Lee, K. H., & Wüthrich, K. (1983) *J. Mol. Biol. 169*, 921-948.
Brown, L. R. (1979) *Biochim. Biophys. Acta 557*, 135-148.
Brown, L. R., & Wüthrich, K. (1981) *Biochim. Biophys. Acta 647*, 95-111.
Brown, L. R., Bösch, C., & Wüthrich, K. (1981) *Biochim. Biophys. Acta 642*, 296-312.
Carnegie, P. R., & Moore, W. J. (1980) in *Proteins of the Nervous System* (Bradshaw, R. A., & Schneider, D. M., Eds.) pp 119-143, Raven Press, New York.
Deber, C. M., & Young, M. E. M. (1979) *J. Biol. Chem. 254*, 6341-6345.
Deber, C. M., Moscarello, M. A., & Wood, D. D. (1978) *Biochemistry 17*, 898-903.
Feigenson, G. W., & Meers, P. R. (1980) *Nature (London) 283*, 313-314.
Gruen, D. W. R. (1981) *J. Colloid Interface Sci. 84*, 281-283.
Keniry, M. A. (1981) Ph.D. Thesis, University of Sydney, Sydney, Australia.
Keniry, M., & Smith, R. (1981) *Biochim. Biophys. Acta 668*, 107-118.
Kira, J., Deibler, G. E., Krutzch, H. C., & Martenson, R. E. (1984) *J. Neurochem.* (in press).
Lauterwein, J., Bösch, C., Brown, L. R., & Wüthrich, K. (1979) *Biochim. Biophys. Acta 556*, 244-264.
Law, M. J., Martenson, R. E., & Deibler, G. E. (1984) *J. Neurochem. 42*, 559-568.
Littlemore, L. A. T., & Ledeen, R. W. (1979) *Aust. J. Chem. 32*, 2631-2636.
Martenson, R. E., Luthy, V., & Deibler, G. E. (1981a) *J. Neurochem. 36*, 58-68.
Martenson, R. E., Law, M. J., Deibler, G. E., & Luthy, V. (1981b) *J. Neurochem. 37*, 1497-1508.
Mendz, G. L., Moore, W. J., & Carnegie, P. R. (1982) *Aust. J. Chem. 35*, 1979-2006.
Mendz, G. L., Moore, W. J., & Martenson, R. E. (1983a) *Biochim. Biophys. Acta 742*, 215-223.
Mendz, G. L., Moore, W. J., & Martenson, R. E. (1983b) *Biochim. Biophys. Acta 748*, 168-175.
Menger, F. M., & Doll, D. W. (1984) *J. Am. Chem. Soc. 106*, 1113-1117.
Nygaard, E., Mendz, G. L., Moore, W. J., & Martenson, R. E. (1984) *Biochemistry 23*, 4003-4010.
Smith, R. (1982) *Biophys. Chem. 16*, 347-354.
Smith, R., Cornell, B. A., Keniry, M. A., & Separovic, R. (1983) *Biochim. Biophys. Acta 732*, 492-498.
Terwillinger, T. C., Weissman, L., & Eisenberg, O. (1982) *Biophys. J. 37*, 353-361.
Wüthrich, K., Bösch, C., & Brown, L. R. (1980) *Biochem. Biophys. Res. Commun. 95*, 1504-1509.

Trinitrobenzenesulfonate Modification of the Lysine Residues in Lactose Repressor Protein†

Peggy A. Whitson, Alex A. Burgum, and Kathleen Shive Matthews*

ABSTRACT: Modification of the lysine residues in the lactose repressor protein has been carried out with trinitrobenzenesulfonate. Reaction of lysine residues at positions 33, 37, 108, 290, and 327 was observed. Inducer binding was increased by modification with this reagent, while both nonspecific DNA binding and operator DNA binding were diminished, although to differing degrees. The loss in operator DNA binding capacity was complete with modification of ~2 equiv of lysine per monomer. The extent of reaction was affected by the presence of both sugar and DNA ligands; binding activities of the modified protein and reaction pattern of the lysines were perturbed by these ligands. The presence of operator or nonspecific DNA during the reaction protected against specific and nonspecific DNA binding activity loss. This protection presumably occurs by steric restriction of reagent access to lysine residues which are essential for both nonspecific and operator binding interactions. Lysines-33 and -108 were protected from modification in the presence of DNA. These experiments suggest that the charge on the lysine residues is important for protein interaction with DNA and that steric constraints for operator DNA interaction with the protein are more restrictive than for nonspecific DNA binding. In contrast, inducer (isopropyl β-D-thiogalactoside) presence partially protected lysine-290 from modification while significantly enhancing reaction at lysine-327. Conformational alterations consequent to inducer binding are apparently reflected in these altered lysine reactivities.

The lactose repressor protein regulates the expression of the genes coding for the *lac* enzymes in *Escherichia coli* by binding with high affinity to the operator sequence in the DNA (Miller & Reznikoff, 1980). The repressor is a tetramer of identical subunits (monomer M_r ~37 500). In the presence of inducer molecules, the affinity of the repressor protein for the operator sequence of the DNA is diminished, and the excess of nonoperator regions in the *E. coli* genome can compete effectively with operator for binding to repressor-inducer complex. The characteristics of the interaction of repressor with inducer, nonspecific DNA, and operator DNA have been widely examined. The affinities of various nonspecific DNAs and operator-containing DNAs have been measured (Miller & Reznikoff, 1980; Winter & von Hippel, 1981); the salt dependence of binding of these different DNA species varies

† From the Department of Biochemistry, Rice University, Houston, Texas 77251. *Received January 31, 1984.* This work was supported by grants from the Robert A. Welch Foundation (C-576), the National Institutes of Health (GM 22441), and the National Science Foundation (PCM 80-12048). The Protein Sequencing Center, University of Texas, Austin, TX, where amino acid analyses were executed, was established in part by NIH Grant GM 21688-05S2 to J. L. Fox.

0006-2960/84/0423-6046$01.50/0