Myelin basic protein is a candidate autoantigen in multiple sclerosis. One of its dominant antigenic epitopes is segment Pro$_{85}$ to Pro$_{96}$ (human sequence numbering, corresponding to Pro$_{82}$ to Pro$_{93}$ in the mouse). There have been several, contradictory predictions of secondary structure in this region; either β-sheet, α-helix, random coil, or combinations thereof have all been proposed. In this paper, molecular dynamics and site-directed spin labeling in aqueous solution indicate that this segment forms a transient α-helix, which is stabilized in 30% trifluoroethanol. When bound to a myelin-like membrane surface, this antigenic segment exhibits a depth profile that is characteristic of an amphipathic α-helix, penetrating up to 12 Å into the bilayer. The α-helix is tilted ∼9°, and the central lysine is in an ideal snorkeling position for side-chain interaction with the negatively charged phospholipid head groups.

Multiple sclerosis (MS) is thought to be an autoimmune disease characterized by chronic inflammatory response against myelin in the central nervous system. There is significant evidence that myelin basic protein (MBP) is a candidate antigen for T-cells and autoantibodies in MS (1). The 18.5-kDa isoform of MBP maintains the compaction of the cytoplasmic face of the two apposing bilayers (2, 3). The human 18.5-kDa isoform of MBP maintains the compaction of the cytoplasmic face of the two apposing bilayers (2, 3). The mechanism and sites that are important for membrane adhesion are not known.

The level of anti-MBP antibodies is increased in the cerebrospinal fluid of patients with active MS (4), as well as in 96% of patients with relapsing and chronic MS (5). An MBP region between Pro$_{85}$ and Pro$_{96}$ (human sequence numbering) was identified to be a minimal B cell epitope and a T cell epitope for HLA DR2b (DRB1*1501)-restricted T cells that recognize the protein (1, 6). This epitope overlaps with the DR2a-restricted epitope for T-cells reactive to MBP residues 87–106 (7). Experimental treatments for MS based on peptide mimetics of MBP have focused on this region of the protein (8).

In solution, MBP is “intrinsically unstructured” (or “natively unfolded”) (9). Upon binding to detergents or lipids, the levels of β-sheet and especially α-helical structure increase dramatically (10, 11). Presently the tertiary structure of MBP is unknown, with the most detailed predictions coming from Martinsson (12) and Stoner (13) in the mid-1980s. In these models, as well as in a newer model based upon further research on an electron microscopy single-particle reconstruction, residues 86–92 are found in a β-sheet (14, 15). Using [3H]NMR and circular dichroic spectropolarimetry of various MBP peptides with detergent micelles, Mendz et al. (16) suggested that there were discrete interaction sites in the protein, one of which could be a helix between residues 87 and 97. Based on the arrangement of hydrophobic and hydrophilic residues, Warren et al. (6) predicted that this epitope of MBP was an amphipathic α-helix located at the interface between the oligodendrocyte cytoplasm and the membrane. In a solution circular dichroism (CD) and Fourier transform infrared spectroscopic study, MBP peptides from this region were found to have both α-helix and β-sheet structures in methanol, with the latter increasing in amount in progressively shorter peptides (17). The crystal structures of peptides 85–99 and 86–105 bound to class II MHC proteins have been found to be extended (18, 19).

We have previously used site-directed spin labeling (SDSL) and electron paramagnetic resonance (EPR) spectroscopy (20, 21) to compare the membrane interactions of normal murine MBP (mMBP) with that of a less cationic form that predominates in MS (22). In that study, H85R1 was found to be motionally restricted, yet situated more than 3 Å above the lipid phosphate groups. Hydrophobicity calculations and helical wheel analysis indicated that the segment surrounding this site could potentially form a surface-seeking amphipathic α-helix. The purpose of this current study was to define more precisely the secondary structure found at this immunodominant epitope when bound to a myelin-like lipid membrane. The previously characterized recombinant murine MBP was used, which has 100% sequence identity with human MBP in this region. The human segment P$_{86}^{85}$-V$_{87}^{89}$-H$_{88}^{86}$-F$_{89}^{90}$-K$_{91}^{90}$-N$_{92}^{91}$-R$_{93}^{92}$-T$_{94}^{93}$-P$_{95}^{94}$ corresponds to murine segment P$_{82}^{81}$-V$_{83}^{85}$-H$_{84}^{82}$-F$_{85}^{86}$-K$_{86}^{85}$-N$_{87}^{86}$-R$_{88}^{87}$-T$_{89}^{88}$-P$_{90}^{89}$ (we shall henceforth refer primarily to the murine sequence numbering). The results ob-
MATERIALS AND METHODS

Site-directed Mutagenesis, Protein Expression, and Purification—The preparation of the Cys-containing mutants was done as described previously (22) using the QuikChange® protocol (Stratagene, La Jolla, CA). Mutant murine MBP expression and purification were carried out as described (11). Spin labeling was done while the protein was bound to the Ni\(^{2+}\)-nitrilotriacetic acid resin, giving rise to the spin-label side chain designated R1.

Preparation of Large Unilamellar Vesicles (LUVs)—Aliquots of the mMBP (83–92) as a semi-stable amphipathic α-helix in solution, which is then stabilized when the protein binds to a membrane, with side chains penetrating up to 12 Å into the bilayer.

EPR Spectroscopy—For signal-averaged EPR spectroscopy, spin-labeled MBP solutions were measured in the presence of 26% sucrose, or 26% sucrose containing 30% trifluoroethanol (TFE), or added to LUVs at a molar protein:lipid ratio of 1:600. This value is slightly less than the MBP:lipid ratio in myelin. For samples in solution, borosilicate glass 50-μl capillary tubes (Fisher) were used to hold the samples. The samples were positioned in the center of the EPR cavity of a Bruker ECS 106 spectrometer (Bruker BioSpin, Milton, ON, Canada), and spectra were recorded at room temperature at a microwave power of 10.0 mW and a modulation amplitude of 1.0 Gauss.

An empirical motion parameter \( \tau_x \) was determined from the first derivatives of the absorption spectra using the following equation

\[
\tau_x = K \Delta H \left( \frac{h_0}{h_{-1}} \right)^{1/2} - 1
\]

where \( K = 6.5 \times 10^{-10} \text{ s} \), \( \Delta H \) is the width of the center-line, and \( h_0 \) and \( h_{-1} \) are the heights of the center and high-field lines, respectively (Fig. 1c, Ref. 22).

When MBP was added to LUVs, aggregation of the vesicles occurred immediately, and after 10 min, the preparations were spun at 1000 \( \times \) g to loosely pellet the MBP-LUV aggregates. The supernatant was removed, and 5 μl of the pellet was loaded into a gas-permeable methylenediphenyl polymer (TPX) capillary. Changes in the mobility of the probe were quantified using the width of the center-line (\( \Delta H \)); a greater \( \Delta H \) value represented a decreased mobility of the spin label (23, 24). Accessibility of the spin-labeled side chains to NiEDDA and O_2 was determined by continuous wave-power saturation as described previously (22) using a Varian E102 Century Series spectrometer (Varian Associates, Palo Alto, CA) equipped with a loop-gap resonator (Medical Advances, Milwaukee, WI). The \( P_{1/2} \) values were determined from saturation curves (21). The \( \Delta P_{1/2} \) values were obtained in the presence of either 20 mM NiEDDA or air (20% O_2), and the depth parameter \( \Phi \) was determined from the natural logarithm of the \( \Delta P_{1/2} \) ratio of O_2 to NiEDDA (21). The normalized accessibility parameter, \( \Pi \), was calculated from \( \Delta P_{1/2} \) as described previously (20).

The dependence of \( \Phi \) on distances from the membrane surface was

FIG. 1. a, amino acid sequence of murine MBP (168 residues) with sites that were mutated to Cys and spin-labeled indicated by shading. b, scheme for the spin-labeling reaction of cysteinyl residues in MBP. The Cys-containing mutants were reacted with the MTS-SL while bound to the Ni\(^{2+}\)-nitrilotriacetic acid resin, giving rise to the spin-label side chain designated R1. c, first derivative EPR spectrum of 1 mg/ml of spin-labeled MBP-H85R1 in aqueous solution (20 mM HEPES-NaOH, pH 7.4, 10 mM NaCl). d, first derivative EPR spectrum of the complex of MBP-H85R1 and Cyt-LUVs. The width of the center-line (\( \Delta H \)), as shown in panels c and d, increases in the presence of lipids, indicating greater motional restriction.

Preparation of Large Unilamellar Vesicles (LUVs)—Aliquots of the Cyt-LUVs. The width of the center-line (\( \Delta H \)), as shown in panels c and d, increases in the presence of lipids, indicating greater motional restriction.

Preparation of Large Unilamellar Vesicles (LUVs)—Aliquots of the Cyt-LUVs. The width of the center-line (\( \Delta H \)), as shown in panels c and d, increases in the presence of lipids, indicating greater motional restriction.
determined using head-group-labeled 1,2-dipalmitoyl-sn-glycero-3-phospho-TEMPO-choline and various PCs with doxyl nitroxides along the acyl chain at positions 5, 7, 10, and 12. The following hyperbolic tangent function of Frazier et al. (25) was used to generate the standard curve for calculating the MBP spin-label depths

\[ \phi = A \tanh[B(x - C)] + D \]  

(Eq. 2) where \( x \) is the distance from the nitrogen of the nitroxide label to the lipid phosphate, and \( A \) and \( D \) define the bulk values of \( \phi \) in water and hydrocarbon, and \( B \) and \( C \) describe the slope of the curve and the inflection point, respectively. This equation was then used to solve for the distance \( x \) using the experimentally derived values of \( \phi \).

Molecular Dynamics—The molecular dynamics simulation of the segment VVHFFK NIVT was performed using AMBER version 6.0 and

| Spin-labeled sample | II (air)\(^a\) | II (NiEDDA)\(^b\) | Distance\(^b\) |
|---------------------|--------------|--------------------|---------------|
| V83R1               | 0.22 ± 0.02  | 0.41 ± 0.05        | -0.60 ± 0.11  |
| V84R1               | 0.25 ± 0.02  | 0.42 ± 0.05        | -0.51 ± 0.10  |
| H85R1               | 0.17 ± 0.02  | 1.19 ± 0.05        | -1.92 ± 0.09  |
| F86R1               | 0.24 ± 0.02  | 1.23 ± 0.05        | -1.65 ± 0.08  |
| F87R1               | 0.29 ± 0.02  | 0.23 ± 0.04        | 0.23 ± 0.15   |
| K88R1               | 0.19 ± 0.02  | 0.61 ± 0.04        | -1.17 ± 0.09  |
| N89R1               | 0.17 ± 0.02  | 0.70 ± 0.05        | -1.42 ± 0.09  |
| I90R1               | 0.22 ± 0.02  | 0.67 ± 0.05        | -0.89 ± 0.08  |
| V91R1               | 0.31 ± 0.02  | 0.20 ± 0.04        | 0.44 ± 0.16   |
| T92R1               | 0.17 ± 0.02  | 0.84 ± 0.05        | -1.57 ± 0.09  |

\( a \) The average standard error associated with II was estimated from replicate experiments.

\( b \) The distances were determined using the fit to Equation 2 using the spin label standards (Fig. 3a).

Fig. 2. \( a \), first derivative EPR spectra of MBP spin-label mutants bound to Cyt-LUVs. The spectra were normalized to the amplitude of the center peak. The black arrows indicate a second, more immobilized component due to the restriction of side-chain mobility because of tertiary contacts or steric interference from other side chains (V84R1 and N89R1). A white arrow indicates spectra that consist of two or three components, one highly mobile and the others corresponding to more immobilized populations (F86R1 and F87R1). \( b \), plot of the inverse of the central EPR line width \( (1/\Delta H) \) for all of the spin-labeled mutants bound to Cyt-LUVs as a function of residue position. The central line width is the width of the peak-to-peak splitting of the first derivative of the \( m_z \) = 0 resonance, with error bars as indicated. The average S.E. associated with the measurement of \( \Delta H \) is ± 0.15 Gauss, as deduced from several replicate experiments. A higher value of \( 1/\Delta H \) indicates greater mobility. In the case of F86R1 and F87R1, the \( \Delta H \) value in the presence of NiEDDA was used, because it removed the mobile component that would artifically reduce the line width. \( c \), accessibility parameters for \( \Delta P_{1/2}(\text{air}) \) (○) and \( \Delta P_{1/2}(\text{NiEDDA}) \) (□) plotted versus residue number. There is a characteristic periodicity indicative of regular secondary structure.

Table I
Collision and depth parameters for putative amphipathic α-helix MBP(83-92)

| Spin-labeled sample | II (air)\(^a\) | II (NiEDDA)\(^b\) | Distance\(^b\) |
|---------------------|--------------|--------------------|---------------|
| V83R1               | 0.22 ± 0.02  | 0.41 ± 0.05        | -0.60 ± 0.11  |
| V84R1               | 0.25 ± 0.02  | 0.42 ± 0.05        | -0.51 ± 0.10  |
| H85R1               | 0.17 ± 0.02  | 1.19 ± 0.05        | -1.92 ± 0.09  |
| F86R1               | 0.24 ± 0.02  | 1.23 ± 0.05        | -1.65 ± 0.08  |
| F87R1               | 0.29 ± 0.02  | 0.23 ± 0.04        | 0.23 ± 0.15   |
| K88R1               | 0.19 ± 0.02  | 0.61 ± 0.04        | -1.17 ± 0.09  |
| N89R1               | 0.17 ± 0.02  | 0.70 ± 0.05        | -1.42 ± 0.09  |
| I90R1               | 0.22 ± 0.02  | 0.67 ± 0.05        | -0.89 ± 0.08  |
| V91R1               | 0.31 ± 0.02  | 0.20 ± 0.04        | 0.44 ± 0.16   |
| T92R1               | 0.17 ± 0.02  | 0.84 ± 0.05        | -1.57 ± 0.09  |

\( a \) The average standard error associated with II was estimated from replicate experiments.

\( b \) The distances were determined using the fit to Equation 2 using the spin label standards (Fig. 3a).
the all-atom force field (26) in a pre-equilibrated water cube with 1300 water molecules and one Cl⁻/H11002 counterion. The system was equilibrated as described previously (27), and a production run of 50 ns was done using a 2-fs time-step. The simulations were run on two SGI Origin 2000 servers (Silicon Graphics Inc., Mountain View, CA) with a total of four processors using the MPI implementation of the SANDER module in AMBER.

Circular Dichroism—CD was performed on a Jasco J-600 spectropolarimeter (Japan Spectroscopic Co., Tokyo, Japan) as described previously (11). The protein concentration used was 0.1 mg/ml in a sample volume of 0.3 ml, in a quartz cuvette with a path-length of 0.1 cm. The measurements were done in the presence of 26% sucrose, or 26% sucrose and 30% TFE to emulate the conditions used for EPR. Measurements were taken over the wavelength range of 260–200 nm. Going further into the far-UV region was not possible, because of the interference of sucrose between 190–200 nm.

RESULTS AND DISCUSSION

mMBP (83–92) Is an Amphipathic α-Helix—Ten consecutive spin-labeled mMBP mutants (V83C, V84C, H85C, F86C, F87C, K88C, N89C, I90C, V91C, T92C) were generated (Fig. 1, a and b). Solution EPR spectra resulted in sharp hyperfine lines, indicating that this region was largely unstructured in aqueous solution (Fig. 1c). When the spin-labeled MBPs were incubated with Cyt-LUVs, which was used to mimic the lipid composition of the myelin sheath, there was almost immediate aggregation of the vesicles, and the spectra became broader and more asymmetric, indicating more restricted anisotropic motion of the spin label (compare Fig. 1, c and d). The aggregation state induced by MBP is a good paradigm for the in vivo situation, because the addition of MBP to lipid vesicles can reform the lipid into stable multilamellar structures similar to the myelin sheath (3, 28).

Upon incubation with Cyt-LUVs (Fig. 2a), the increase of the central line width (ΔH) can be used as a measure of the tumbling rate of the nitroxide. The plot of 1/ΔH as a function of residue number reveals a periodic variation in motion (Fig. 2b). Continuous wave-power saturation EPR spectroscopy was used to determine the accessibility of these 10 residues when bound to the vesicles. The water-soluble paramagnetic reagent nickel-ethylenediaminediacetic acid (NiEDDA) was

![Figure 3](image-url)

**Fig. 3. Depth measurement of spin labels into Cyt-LUVs via power saturation.** a, calibration curve for doxyl spin-labeled lipids using Cyt-LUVs (44 mol % cholesterol, 27 mol % PE, 13 mol % phosphatidylserine, 11 mol % PG, 3 mol % sphingomyelin, 2 mol % PI). Closed circles represent the Φ values in the presence of 180 μg of unlabeled MBP with 1 mg of Cyt-LUVs. The curve shows the fit to Equation 2. The curve was constrained by the aqueous bulk limit (Φ = –2.6) and the upper Φ limit of the membrane interior (Φ = –4.5) as described (25). The parameters from the best fit for the various spin-labeled PCs with the Cyt-LUV lipid mixture with bound, unlabeled MBP were: A = 3.2; B = –0.15; C = 12.0; and D = 1.3. The red circles represent the data for spin-labeled MBPs that were fitted to the hyperbolic tangent function (Equation 2). b, depths of penetration of all MBP spin labels into the lipid bilayer of the Cyt-LUVs (red circles). The gray shading indicates values below the lipid phosphates (in the bilayer). The amphipathic helix from the molecular dynamics simulation (c) and the peptide structure from the co-crystal of MBP (84–102) with the MHC II molecule modeled within a simulated bilayer (d). The generated spin-label depths for the crystal structure of the peptide were plotted on the depth plot (blue circles in b) to show that this particular conformation could not give rise to the spin label depths obtained. ε, a schematic representation of the depth of penetration of the helix with the most exposed (His-85) and deepest penetrating residues (Phe-87) indicated. The hydrocarbon region starts ~7 Å from the lipid-water interface. Lys-88 is shown in a snorkeling orientation interacting with the negative phosphate group of a POPC phospholipid.
used to probe the solvent accessibility ($\Pi_{\text{NiEDDA}}$), and nonpolar molecular oxygen was used to determine the insertion of the nitroxide into the hydrophobic environment of the bilayer ($\Pi_{\text{O}_2}$) (Table I).

The plot of $\Pi_{\text{NiEDDA}}$ and $\Pi_{\text{O}_2}$ as a function of residue number indicated that as the accessibility to NiEDDA increased, the accessibility to $\text{O}_2$ decreased, and vice versa (Fig. 2c). Moreover, this pattern occurred in a periodic manner indicative of regular secondary structure. The accessibility parameter, $\Phi$, was determined from the natural logarithm of the ratio of $\Pi_{\text{O}_2}$ to $\Pi_{\text{NiEDDA}}$. A standard curve of known immersion depths was generated using various spin-labeled lipids. The calibration of the $\Phi$ value with the depth of penetration was especially important for the Cyt-LUVs, because they consisted of six different lipid components, including 40% cholesterol. The effect of the cholesterol was to increase the penetration of NiEDDA up to the fifth carbon of the acyl group of the phospholipid, as reported previously (22). The hyperbolic tangent function (Eq. 2) of Frazier et al. (25) was used to fit the data and calculate the depth of penetration of the residues into the membrane (Fig. 3, a and b).

The immersion depths for the segment mMBP (83–92) showed a periodic oscillation indicative of an $\alpha$-helix. The data were fitted to a sine function with a periodicity of 3.6 residues, representing the geometry of a model $\alpha$-helix. Because the residues penetrated deeper into the membrane toward the C terminus, indicating that the helix was tilted, an extra variable was added to the sine function to take into account the angle of the tilt (29, 30). The experimentally determined depths fit well to the modified sine function (Fig. 3b). The putative helix had

**FIG. 4. Molecular dynamics simulation of the MBP (83–92) peptide in aqueous solution with added counterion.** The starting configuration was the structure in complex with an MHC protein (Protein Data Bank ID code 1bx2). a, snapshots of the simulation from 1 to 50 ns. b, plots of the radius of gyration (red line) and the root mean square difference with respect to the starting conformation (blue line) over time.
an amplitude (diameter) of 12–13 Å, which is consistent with the diameter of an α-helix bearing a nitroxide arm (29), and it was tilted in the bilayer at an angle of −9° with respect to the membrane plane.

**Molecular Dynamics of Val83-Thr92**—Using the structure of the human MBP peptide that was co-crystallized with the human histocompatibility leukocyte antigen HLA-DR2 as a starting conformation (Protein Data Bank ID code 1bx2), a molecular dynamics simulation was done in water with an added chlorine counterion to neutralize the single lysyl residue. The simulation employed an empirical force field to describe the energy of the system as a sophisticated sum of bond, angle, torsion, van der Waals and electrostatic interaction energies. The classical Newtonian equations of motion were integrated at specific time steps to give the time evolution of the system, which, in this study, went to 50 ns.

The original peptide was in an extended conformation; however, a series of snapshots of the structure at 10-ns intervals showed the variability of the structure and its tendency to form an α-helix (Fig. 4a). At 30 ns, the entire peptide formed a conventional α-helix, but 10 ns later, the amount of random coil had increased, and at 50 ns, the entire regular structure had dissipated. After only the first few ps of the simulation, there was an immediate increase in the root mean square difference from the initial structure (Fig. 4b). After reaching 4 Å, the root mean square difference varied between 3 and 6 Å for the rest of the simulation. The radius of gyration ($R_g$) also fluctuated steadily between 8 and 6 Å, representing more extended and more compacted states, respectively (Fig. 4b). In summary, these simulations indicate that the segment mMBP (83–92) has a propensity to form an α-helix in aqueous solution but that this structure is transient in the absence of any stabilizing factors.

**Comparison with Crystal Structure of this MBP Epitope Complexed with HLA-DR2**—The crystal structures for soluble DRB1*1501-hMBP (85–99) (18) and DRB5*0101-hMBP (86–105) (31) complexes have been reported. Both peptides are extended, linear fragments, with little evidence of ordered secondary structure. To see whether it might be possible for the extended structure from the DRB1*1501-hMBP (85–99) complex to fit the accessibility data obtained for this region of MBP bound to lipid, the extended structure was compared with the helical peptide structure obtained after 30 ns of molecular dynamics (Fig. 3, c and d). Both peptides were tilted at an angle
of 9° to determine which one fit the depth measurements from SDS-PAGE. The modeling was done so that the most accessible residue (His95) was exposed to the aqueous phase, whereas the most deeply penetrating residues (Val99 and Phe100) faced the hydrophobic core of the bilayer. This geometry fell readily into place for the α-helical peptide, because of the natural arrangement of the hydrophobic and hydrophilic residues on opposite sides. However, for the extended structure, His95 and Phe100 pointed in almost the same direction, making an orientation that accurately fit the data very difficult to determine. The closest match possible is shown in Fig. 3e. The predicted accessibilities from the α-helix determined from molecular dynamics fit the experimental data almost perfectly, whereas the majority of the crystal fragment residues did not fit (Fig. 3f, blue circles). This result was not unexpected, because the MHC II molecule imposes on its targets a conformation dependent upon the structure of the MHC itself.

Circular Dichroism and EPR of mMBP (83–92) with TFE—To evaluate experimentally the question of whether the putative α-helix was stable in solution, EPR was done in the presence of 26% sucrose to slow down the tumbling motion of MBPs and to aid in distinguishing the local mobility of the spin label from that of the peptide backbone (32). The spectra of the spin-labeled mutants were significantly more asymmetric with sucrose present, and the asymmetry increased further in the presence of TFE (Fig. 5a). The empirical motion parameter (τ2) indicated that mMBP (83–92) was more restricted in the presence of TFE, and thus that there was more ordered structure in this region (Fig. 5b). The periodicity of variations in the motion fit a sine function with a periodicity of 3.6 residues, which is consistent with an α-helical structure.

Mchourab et al. (24) observed a similar periodic oscillation for an α-helical segment of lysozyme. They showed that the motion of R1 on a helix surface was restricted relative to that on accessible loops, because of hindrance of rotational motion of the spin label moiety about the disulfide bond and the Ca-Cβ bond of cysteine. This effect was due to interference of the cysteine β carbon with the backbone of the preceding turn and by interaction of the disulfide moiety with the protein backbone. They also showed that the motion of the spin label reflected the protein backbone motion, not steric interference by other helix residue side chains. Here, the motions of all spin-labeled residues of the mMBP (83–92) segment in sucrose and TFE/sucrose were significantly greater than those for lysozyme (of comparable molecular mass) in sucrose. This comparison indicates a greater backbone motion for MBP because of its lack of tertiary structure in solution, in contrast to lysozyme with a globular tertiary structure, which significantly reduces thermal fluctuations of the backbone in most regions.

The overall effect of TFE on MBP structure was also investigated using CD. All 10 spin-labeled proteins used in this study were observed to behave the same, despite the amino acid substitutions. The samples in sucrose alone all had a spectrum characteristic of an unstructured protein (Fig. 5c). In the presence of TFE, the proportion of α-helical structure increased dramatically, as evidenced by the troughs in the spectra at 208 and 222 nm (Fig. 5d) (33). In summary, the explanation for both the EPR and CD observations is that TFE provides a low dielectric environment that favors the formation of intrapeptide hydrogen bonds, thus stabilizing the α-helix formed by mMBP (83–92) (34).

Hemi-fusion Paradigm for Membrane Adhesion—The EPR, molecular dynamics, and CD results all complement one another and show that the conformation of segment mMBP (83–92) is highly dependent upon its environment. The absence of stable structure in aqueous solution was simulated computationally and borne out experimentally by EPR and CD spectroscopy. In comparison, SDS analysis showed that when MBP was bound to the lipid bilayer, the helical structure in this region was stabilized.

How MBP brings together multiple vesicles in vitro (and in vivo in the myelin sheath) is an important biological question. The epitope helix found here is undoubtedly one of many of MBP that are involved in the adhesion of lipid bilayers in myelin. Previous studies have shown that MBP causes the hemi-fusion of membranes and does not allow mixing of the vesicle compartments (35). In addition, the α-helical epitope from mMBP (83–92) is tilted with respect to the membrane plane, similar to that seen for other fusion proteins (36). This tilt is a result of the larger hydrophobic potential profile around the C-terminal end of the helix. In this case, the tilt is only 9°, as compared with the hemagglutinin fusion peptide, which is tilted 25° (29). Thus the degree of tilt for MBP is sufficient for deep penetration and firm membrane adhesion, but is not large enough to destabilize the bilayer. We have shown that deletion mutants of both the N and C termini of MBP still had the ability to aggregate vesicles, although at lower efficiency (37). Both deletion mutants contained the 83–92 segment studied here, indicating that it may be an essential component in the MBP-mediated adhesion of bilayers.

The observation that K88R1 is the deepest penetrating polar residue in this study (5.5 Å into the bilayer) suggests that it could be involved in snorkeling, whereby the long side chain would be bent to place the charged amino group in the more polar interface region, while keeping the hydrocarbon part of the side chain inside the hydrophobic part of the membrane (38). The orientation of K88R1 as shown in Fig. 3e indicates that the positive side chain could be positioned for electrostatic interaction with the phosphate group of the phospholipid, which could contribute to tighter binding of the protein to the membrane.

In summary, the structure of MBP is still unknown despite numerous attempts using a variety of techniques and methodologies (9). Here, the SDS-ESR technique was used to resolve the structure of an immunodominant epitope of MBP (murine segment 83–92 corresponding to the human segment 86–95) in a natural environment, showing it to be an amphipathic α-helix with a tilted arrangement similar to that of a fusion peptide. The topology of MBP bound to the membrane shows that key contact points for antibody recognition, F86, F87, K88 (F89, F90, K91 in the human), are buried in the lipid bilayer and thus would be relatively sequestered from other proteins. Antibody binding to membrane-bound MBP would occur only if this region transiently dissociates from the membrane.

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**Structure of an Antigenic Epitope of MBP**