tion of 0.1 M $MgSO_4$, the spectrum did change. The CD spectrum which was then measured was in between that of native T4 DNA and the spectrum of the complex measured at 1 °C directly after mixing. Apparently the protein DNA complex dissociated partially and renaturation to almost completely double stranded molecules occurred.

Comparison with gp32 Complexation. From the data presented above and the data given in the preceding paper (Greve et al., 1978) it follows that gp32*I has a slightly different action than gp32. Firstly gp32*I shifts the melting temperature of T4 DNA by about 60 °C so that under physiological conditions the T4 DNA is strand separated. Secondly in the interaction with poly[d(A-T)]·poly[d(A-T)] complexes are already formed at 1 °C and complete strand separation occurs (at high protein concentration) at about 10 °C. This is about 15 °C lower than in the case with gp32. Thirdly the interaction with gp32*I seems to result in a single-stranded poly[d(A-T)] structure which is slightly different.

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Conformational Studies on ^{13}C-Enriched Human and Bovine Myelin Basic Protein, in Solution and Incorporated into Liposomes†

C. M. Deber,* M. A. Moscarello, and D. D. Wood

ABSTRACT: Carbon-13 nuclear magnetic resonance (NMR) spin-lattice relaxation (T_1) and line-width studies have been performed on human and bovine myelin basic proteins in solution and incorporated into liposomes. These studies were facilitated by carbon-13 enrichment of the two methionyl residues in the protein which provided an S-methylated form and a noncovalently modified "intact" methionine carbon-13 enriched form. Values of T_1 for side chain methionine S-CH_3 carbons, in solution, found to be 0.5 to 0.6 s for the S-methylated proteins, and 0.7 to 0.8 s for the intact protein, displayed no significant variation over a range of pH or protein concentration. Line widths of corresponding spectra were essentially constant. The data were interpreted in terms of a relatively loose overall structure for the protein in which local (segmental) motion, at least in the vicinity of methionyl residues, dominated relaxation phenomena. Experiments were performed wherein ^{13}C-enriched myelin basic protein (both in the S-methylated form and the intact form) was incorporated into lipid vesicles (50:50 phosphatidylcholine:phosphatidic acid containing 30% protein by weight). Carbon-13 enriched Met S-CH_3 resonances corresponding to the protein interacting with the liposome were clearly visible and displayed line widths of about 15 Hz, as compared with about 1 Hz for the protein in free solution.

Myelin basic protein, a principal protein component of the myelin sheath, has been studied by several biophysical techniques with the goal of elucidating its structure and conformation and ultimately its function in the myelin membrane. Studies have utilized optical rotatory dispersion (ORD)[1] and circular dichroism (CD) (Eylar & Thompson, 1969; Palmer & Dawson, 1969; Chao & Einstein, 1970), intrinsic viscosity (Eylar & Thompson, 1969; Chao & Einstein, 1970; Epand, et al., 1974), low angle x-ray scattering (Epand et al., 1974), surface tension (Moscarello et al., 1974), proton magnetic resonance (Block et al., 1973; Liebes et al., 1975), and natural abundance ^{13}C NMR (Chapman & Moore, 1976). Despite the application of these diverse techniques, the detailed structure of this protein remains to be elucidated.

Although ORD/CD studies showed that no α-helical or β-structure was present in the molecule (Eylar & Thompson, 1969; Chao & Einstein, 1970; Palmer & Dawson, 1969), a specific tertiary structure was postulated on the basis of intrinsic viscosity and low angle x-ray scattering measurements (Epand et al., 1974). From the latter, the dimensions of the protein were calculated to be 15 × 150 Å, representative of a prolate ellipsoid. Evidence for the presence of a nonrandom conformation has also been presented by Chapman & Moore (1976), using natural abundance ^{13}C NMR. Their results suggested structured regions near the middle of the polypeptide chain, a sequence which includes a proline-rich region which could impart a "hair-pin" bend to the molecule (Eylar, 1970). Such a structure could leave both the N-terminal and the C-terminal ends relatively less rigid, and readily able to adopt a conformation in response to environmental constraints as might be expected to occur after interaction of this molecule with a lipid bilayer. Hence, knowledge of the structure of these ter-

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[1] Abbreviations used: NMR, nuclear magnetic resonance; MBP, myelin basic protein; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

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minal regions is clearly desirable.

Although bovine MBP has been used in most investigations to date, this study focuses also on the properties of human MBP. The human protein amino acid sequence differs from the bovine only in 11 substitutions along the chain, and an insert of His-Gly between residues 10 and 11, giving human MBP 172 residues vs. 170 for bovine (Eylar, 1970). In the present manuscript, we sought to gain further insights into the conformational behavior of these proteins, through a carbon-13 NMR study of spectral parameters derived from carbon-13 enriched samples of these materials. In addition, the initial results of carbon-13 NMR experiments examining myelin basic protein incorporated into lipid vesicles are presented.

Materials and Methods

Materials. Bovine myelin basic protein was the generous gift of Dr. E. H. Eylar, Playfair Neurosciences Institute, Toronto. Human myelin basic protein was obtained from normal human white matter and purified by the method of Lowden et al. (1966). ^{13}C-labeled (90% enriched) methyl iodide was obtained from Merck, Sharp and Dohme, Montreal; [^{14}C]-methyl iodide was purchased from New England Nuclear, Cambridge, Mass. Egg phosphatidylcholine was purchased from Serdary Research Laboratories (London, Ontario) and was chromatographically pure. Egg phosphatidic acid was obtained from Dr. D. Papahadjopoulos, Buffalo, N.Y.

Carbon-13 Labeling of Myelin Basic Protein. Methylation of MBP with ^{13}C-enriched methyl iodide was carried out, with minor modifications, according to the procedure given by Jones et al. (1975, 1976). Lyophilized bovine myelin basic protein (6.25 μmol) was dissolved in 10 mL of 0.1 M KNO_3 and the pH was adjusted to 4.0 with 0.1 N HCl. To this was added a 150 molar excess of a mixture of carbon-13 and carbon-14 enriched methyl iodide (8.4×10^2 μmol). The solution was stirred at room temperature in the dark for 24 h. The reaction mixture was dialyzed initially against 0.02% sodium azide and then distilled water. The extent of methylation of the two methionyl residues of MBP was calculated as follows: the total radioactivity of the methylated MBP solution was 1.56×10^6 dpm, with a specific activity of 1.4×10^4 dpm/mg protein. This solution contained 6.25 μmol of protein, or 12.5 μmol of methionine. Since 8.4×10^2 μmol of the [^{13}C, ^{14}C]-enriched methyl iodide was found to have a total radioactivity equal to 1.11×10^8 dpm, incorporation of 12.5 μmol of methyl iodide into MBP would give a radioactivity of 1.6×10^6 dpm. Thus, this experiment yields myelin basic protein which is methylated at both methionines to a total extent of 1.56/1.6 = 97.5%.

For NMR experiments, these protein solutions were lyophilized, dissolved in D_2O, and adjusted with 1 N HCl to the desired pH value.

Reduction of S-Methylated Basic Protein to the Native Protein. A sample of methylated bovine myelin basic protein was diluted to 6 mL with distilled water, the solution was cooled to 0 °C, and the pH adjusted to 10.5 with 5 N NaOH. Dithiothreitol was added dropwise over a 30-min period (Jones et al., 1976), while the pH was maintained between 9.5 and 11.5 by intermittent addition of 5 N NaOH to a final dithiothreitol concentration of 0.5 M, and a final pH of 10.5. The solution was maintained at 37 °C for 18 h and then dialyzed against 0.02% sodium azide and then extensively against distilled water. The extent of reduction of S-methylated MBP to "intact" MBP was generally ascertained by monitoring the difference in specific activity between the protein sample before and after reduction.

Complete reduction of fully methylated MBP (based on a random loss of $^{12}CH_3$, $^{13}CH_3$, or $^{14}CH_3$) corresponds to a loss of 50% of original specific activity. In a typical (100 mg of protein) preparation for use in NMR experiments, S-methylated MBP having specific activity of 1.7×10^4 dpm/mg was converted, upon reduction, to intact MBP having specific activity 8.4×10^3 dpm/mg (vs. the theoretical value of 8.5×10^3 dpm/mg).

Incorporation of Myelin Basic Protein into Vesicles. Lyophilized [^{13}C, ^{14}C]-enriched bovine MBP (25 mg) was taken up in 5 mL of 2-chloroethanol and then sonicated (bath-type sonicator, Bransonic Inc.) until the solution clarified. A mixture (50:50 w/w) of egg phosphatidylcholine (30 mg) and egg phosphatidic acid (30 mg) in 5 mL of 2-chloroethanol solution was prepared and added to the protein solution. The resulting combined material was dialyzed vs. 6 L of vesicle buffer (10 mM Na^+, 2 mM Hepes, pH 7.4) by the method of Boggs & Moscarello (1977). The retentate was centrifuged at 12 000 rpm for 5 min at room temperature. The resulting pellet was suspended in 1.4 mL of vesicle buffer (as above, but prepared in D_2O) for use in NMR experiments.

NMR Methods. Carbon-13 nuclear magnetic resonance spectra reported herein were recorded on a Varian XL-100 spectrometer at 25.16 MHz. Spectral parameters are given in figure legends for individual protein spectra. Spin–lattice relaxation times (T_1's) were measured using the inversion–recovery method which utilizes a $-(180°\text{-}t\text{-}90°\text{-}nT_1)$ sequence (Freeman & Hill, 1971). In the present experiments, T_1's on enriched S-CH_3 Met carbons were generally obtained from 7 to 10 points per experiment, with values of t, the variable time interval, ranging between 10 and 1500 ms, and a typical pulse delay (nT_1) equal to 2 s. ^{13}C-enriched S-CH_3 resonances could generally be visualized after 1000 to 2500 accumulations per point. Acquisition times were usually 0.5 s, spectrum width usually 4000 Hz. Ambient probe temperature was 30 ± 2 °C. Raw spectra were processed in two ways: (1) peak heights were measured directly; and (2) peak weights were obtained by cutting out and weighing chart paper; in the latter case, each peak was weighed twice and the weights averaged. Each reported T_1 value is the average of height and weight determinations. A computer program utilizing a nonlinear least-squares fitting procedure was employed to compute T_1's. Estimated error of reported T_1 values is ±10%.

Line widths reported are the results of measurements of the widths at half-height of indicated resonances, taken directly from expanded plots of experimental spectra. Values given are already adjusted for broadening wherever sensitivity enhancement (SE, given in seconds) was required during data processing; this was performed by subtracting a factor of $1/(\pi \times \text{SE})$ from the observed line widths.

Results

Preparation of Carbon-13 Enriched Samples of Bovine and Human Myelin Basic Proteins. An examination of the sequence of both human and bovine myelin basic protein shows that two methionines are present, at positions 20 and 167 from the N-terminal end (Met positions given throughout are for bovine MBP; corresponding values are 22 and 169 for human MBP) and the molecule is devoid of cysteine residues. Myelin basic protein is therefore an excellent molecule for specific ^{13}C enrichment of the methionyl residues (Jones et al., 1975, 1976), via labeling with $^{13}CH_3I$. As shown in Figure 1, the procedure yields initially the S-methylated methionine derivative, which upon subsequent reduction with dithiothreitol, is restored to the original "intact" methionine material. The resulting protein is thus not altered covalently and is up to 50% enriched at the S-CH_3 methionine positions.

We have now prepared both completely methylated bovine

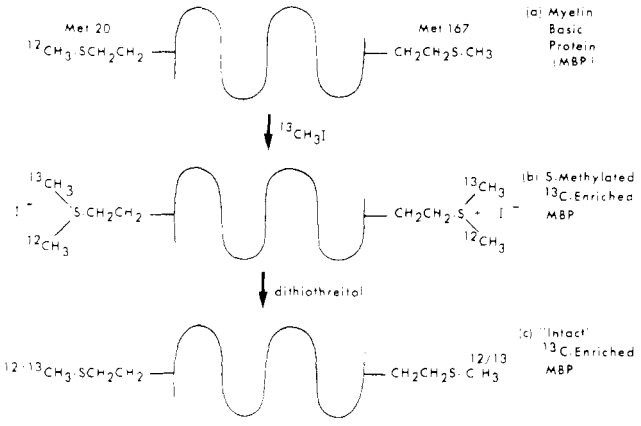

FIGURE 1: Methylation of myelin basic protein with carbon-13 enriched methyl iodide ($^{13}CH_3I$). The natural protein, depicted in a, has two methionine sites which are converted to *S*-methylmethionines in b. Subsequent reduction with dithiothreitol produces "intact" ^{13}C-labeled protein, as shown in c. The shape of the protein chain is intended only as a schematic and has no conformational implications.

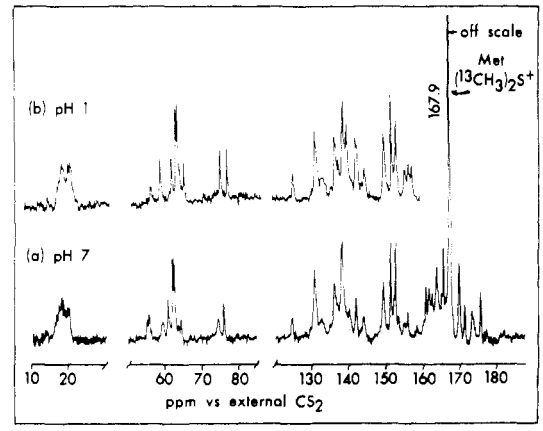

FIGURE 2: Carbon-13 NMR spectra (25 MHz) of S-methylated ^{13}C-enriched bovine myelin basic protein at (a) pH 7 and (b) pH 1. Both samples displayed the enriched *S*-methyl resonance at 167.9 ppm. Spectra are the result of approximately 100 000 scans, using acquisition time of 0.5 s, spectrum width of 5000 Hz, and sensitivity enhancement in data processing of 0.3 s. Concentration was 70 mg/mL in D_2O. The spectral region around 20 ppm contains the carbonyl resonances; the region between 55 and 75 contains the aromatic resonances; that between 130 and 140 contains mainly α-carbon resonances (except Gly α carbons which are near 150 ppm); and the region above 145 ppm contains mainly side chain methyl and methylene carbons. Two small resonances near 36 ppm (probably quaternary carbons such as the Arg guanidinium carbon) have been omitted from the diagrams. A perceptible sharpening of the spectra, particularly in the aromatic region, and somewhat in the α-carbon region, is observed, similar to that noted by Chapman & Moore (1976) for natural abundance unmodified bovine MBP at 200 mg/mL. Chemical shifts may be converted to values relative to external tetramethylsilane by subtracting the values given from 193.8 ppm. The portion of spectrum b not shown in the figure was virtually identical with the corresponding portion of a.

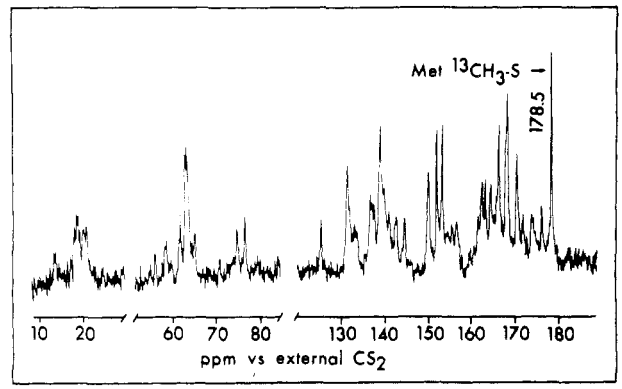

FIGURE 3: Carbon-13 NMR spectra (25 MHz) of intact ^{13}C-enriched human myelin basic protein at pH 5.5. Concentration was 70 mg/mL in D_2O. Spectrum is the result of about 100 000 scans, using an acquisition time of 0.5 s, a spectrum width of 5000 Hz, and a sensitivity enhancement during data processing of 0.2 s. The resonance at 178.5 ppm represents the ^{13}C-enriched *S*-methylmethionine carbon. Further information is given in the caption to Figure 2.

and human myelin basic protein and the corresponding completely reduced proteins, in which both (^{13}C-enriched) Met residues have been restored to their original intact forms. These experiments were performed using methyl iodide which was labeled also with carbon-14, thus allowing us to monitor the progress of the reactions by incorporation of radioactivity.[2]

^{13}C-NMR Spectra of Bovine and Human Myelin Basic Proteins. If MBP possessed any of the usual categories of ordered secondary structure in solution near physiological pH values, this ought to be destroyed via denaturation of the protein at acidic pH (about 1). Such a conformational transition should be manifested in experimental carbon-13 spectra by sharpening of spectral peaks (i.e., narrowing of line widths) (Gurd & Keim, 1973; Doddrell et al., 1972) and by a significant increase in T_1 spin-lattice relaxation values accompanying the expected increase in internal or segmental motion of local portions of the polypeptide chain. In this context, we examined several pairs of ^{13}C spectra; both human and bovine MBP were studied at pH 7 (or just below) and pH 1. NMR sample solutions of human MBP displayed considerable turbidity above pH 5–6, and all protein solutions of human MBP precipitated due to aggregation above pH 7, thus precluding NMR measurements at higher pH values.

Typical ^{13}C NMR spectra of myelin basic protein enriched with ^{13}C, as described in Materials and Methods, are shown in Figure 2. Spectra of both human and bovine MBP, S-methylated with ^{13}C-enriched methyl iodide, gave an intense single resonance at 167.9 ppm (vs. external CS_2), while the corresponding reduced ("intact") protein samples (e.g., Figure 3) gave a single (enriched) Met S–CH_3 about 10 ppm upfield (at 178.5 ppm) in accord with the upfield shift expected upon demethylation of the positively charged $S^+(CH_3)_2$ moiety.

T_1 Measurements on Myelin Basic Protein. Spin-lattice relaxation values (T_1's) for S–CH_3 carbons in bovine and human MBP are reported in Table I. In the S-methylated forms, bovine and human MBP behave similarly, displaying T_1's between 0.5 and 0.6 s, essentially independent of pH. The greater T_1 values (0.74 and 0.83 s) obtained for the "intact" human MBP are likely a consequence of the fact that the intact Met residue is an "*S*-monomethyl" species as opposed to an "*S*-dimethyl" species in the S-methylated cases. Rotation of the latter species about the C_β–S axis would be inherently slower than the former due to increased molecular bulk. This effect has been observed for branched methyl groups attached to ends of hydrocarbon chains (Lyerla et al., 1974).

Line-Width Measurements. Accurate measurement of line widths is complicated by several general factors, including

[2] In a further experiment to determine the distribution of labeling between the Met-20 and Met-167 residues, a sample of S-methylated (^{13}C, ^{14}C-enriched) bovine basic protein (which contains a single tryptophanyl residue at position 116) was cleaved specifically at the Trp-Gly peptide bond with BNPS-skatole (Fontana et al., 1973). After cleavage, two peptides were separated upon passage through two Sephadex G-50 columns which displayed the expected amino acid analyses, and comparable specific activities (1035 dpm/μmol of amino acid for peptide 1–116; 1605 dpm/μmol of amino acid for peptide 117–170).

TABLE I: Spin-Lattice Relaxation Values (T_1's) for S-CH_3 Methionine Carbons in ^{13}C-Enriched Myelin Basic Protein.

MBP[a] source	Chemical state of Met	pH[b]	Concn (mg/mL)	T_1 (s)[c]
Bovine	S-methylated	7	70[d]	0.56
Bovine	S-methylated	1	70[d]	0.52
Human	S-methylated	6.5	80	0.50
Human	S-methylated	1	80	0.60
Human	Intact[e]	5.5	70	0.74
Human	Intact[e]	1	70	0.83

[a] MBP = myelin basic protein. [b] pH values are uncorrected for D_2O. Human MBP samples could not be run at pH higher than 6.5 or 5.5 in samples above due to low solubility. [c] Reported T_1 values obtained from D_2O solutions at the indicated concentrations and pH values. Data processed as described in Methods. Estimated uncertainty in T_1's: ±10%. [d] A set of experiments with S-methylated bovine MBP at a concentration of 110 mg/mL (at pH 7 and 1) yielded similar T_1's. [e] Sample obtained from dithiothreitol reduction of S-methylated derivative consists of intact human MBP with methionine S-CH_3 about 50% enriched with ^{13}C.

instrument inhomogeneities, incomplete carbon-hydrogen decoupling and/or peak broadening during data processing. Further, in the protein spectra presented here, two additional specific factors must be considered: chemical shift heterogeneity (due to the fact that the ^{13}C-enriched resonance results from two potentially nonequivalent Met residues), and the possibility of protein aggregation (which is observed for MBP under certain conditions) (Liebes et al., 1975).

When the spectrum of the amino acid methionine was recorded under (the nonoptimal) instrumental conditions similar to those used for the protein spectra (e.g., 80 mg/mL, pH 1, 5000 Hz spectrum width), an S-CH_3 Met resonance line width of about 4 Hz was obtained (in contrast to the actual "natural" line width which is likely on the order of a fraction of a hertz). Since the protein line widths measured experimentally were found to be of the order of 4.5–5.4 Hz, including all samples investigated, instrumental inhomogeneity did not place a lower limit on line widths observed in protein spectra. If a "correction factor" of 4 Hz is subtracted from experimentally observed protein line widths, values for $S^+(CH_3)_2$ and S-CH_3 resonances range between 0.5 and 1.4 Hz. Line widths of ^{13}C-enriched methionine resonances of human and bovine MBP were found to remain relatively constant from one sample to another. This constancy, and the sharpness of the spectra in general, suggest that chemical shift heterogeneity (e.g., of the two Met residues) does not contribute significantly to line width.

The possibility of some line broadening due to aggregation was examined for the case of bovine MBP at pH 7, where the spectrum was recorded at lower concentration than other samples (approximately 8 mg/mL instead of 70–80 mg/mL). At this concentration level, it was possible to visualize the ^{13}C-enriched resonance with about 150 000 accumulations. The line width of this resonance remained the same as those observed for 70–80 mg/mL solutions.

Incorporation of Myelin Basic Protein into Liposomes. ^{13}C-enriched S-methylated and intact bovine myelin basic proteins (30% by weight) were incorporated into liposomes consisting of a 50:50 mixture of phosphatidylcholine and phosphatidic acid as described in Materials and Methods. This incorporation procedure has been shown, principally by the techniques of electron spin resonance and differential scanning calorimetry (Boggs & Moscarello, 1977; Boggs et al., 1978),

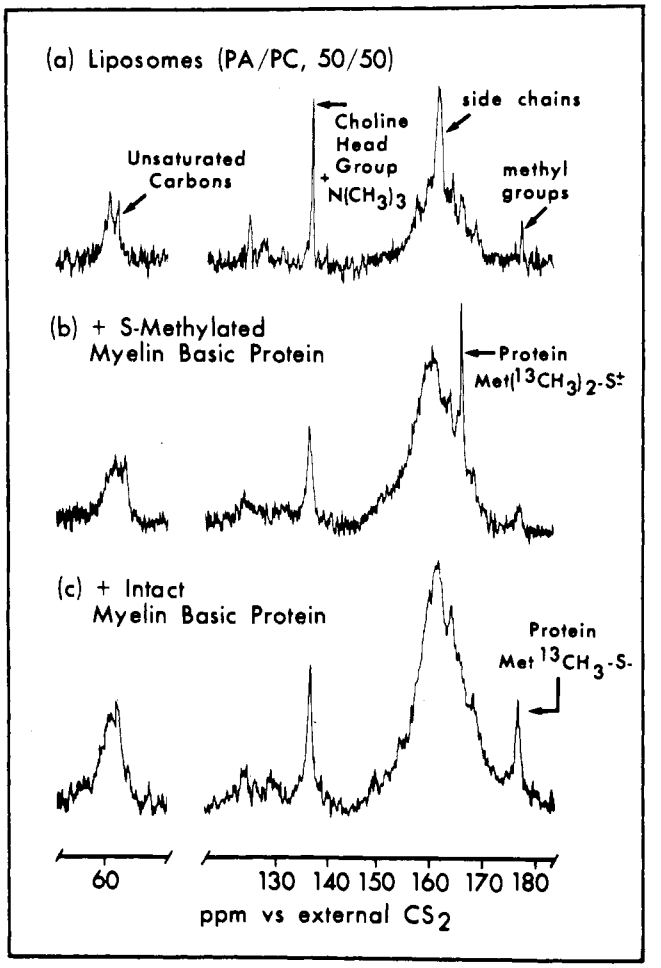

FIGURE 4: Incorporation of S-methylated and intact ^{13}C-enriched bovine myelin basic protein into lipid vesicles. The carbon-13 spectra shown are from samples containing approximately (a) 45 mg of lipids (50/50 w/w phosphatidylcholine and phosphatidic acid) suspended in 1.4 mL of D_2O vesicle buffer (2 mM Hepes, 10 mM Na^+, pH 7.4); (b) and (c), 60 mg of lipids plus 20 mg of S-methylated and intact MBP, respectively. Spectra are the result of 110 000 to 140 000 scans, acquisition time 0.5 s, spectrum width 5000 Hz, and sensitivity enhancement of 0.2 s in a and b and 0.1 s in c. Lipid carbonyl carbons were also visible at lower field. Resonance assignments are as shown in the figure. See text for further discussion of these spectra.

to produce a liposome into which myelin basic protein is embedded, interacting largely with phospholipid polar head groups, but also penetrating part of the way into the bilayer interior.

Preliminary results of these experiments are presented in Figure 4. Spectrum 4a shows the phosphatidic acid/phosphatidylcholine (PA/PC, 50/50 w/w) liposomes alone, at the same concentration as used when the protein was added. Assignments of lipid carbons are the same as those given by Brown & Wüthrich (1977) in a study of the interactions of cytochrome *c* with mixed cardiolipin/phosphatidylcholine vesicles. When S-methylated, ^{13}C-enriched bovine myelin basic protein is incorporated (Figure 4b), the enriched protein $S^+(CH_3)_2$ methyl carbon resonance is seen at 167.8 ppm as a peak superimposed upon the broad resonance due to lipid fatty acid side chains. Lipid resonances in spectrum 4b are obviously broadened vs. those in the free liposome spectrum. Intact, ^{13}C-enriched bovine MBP was incorporated into liposomes in an identical manner; the resulting spectrum (Figure 4c) shows the enriched resonance at the expected position near 178.5 ppm. Both forms of the protein appear to have compa-

rable effects on the liposomes, and this observation, coupled with parallel behavior of several spectral parameters described above, suggests that the methylated protein would serve as a reasonable model for intact MBP in many experiments.

Discussion

From the data presented above (as reflected in Figures 2 and 3, and Table I), measurements of T_1 spin–lattice relaxation times, and of line widths of the ^{13}C-enriched resonances, indicate that the Met residues of MBP are not in regions of MBP which undergo pH-dependent "denaturation", and that the S-CH_3 substituents exhibit a high degree of local rotational freedom. The combination of experimental values obtained for T_1's and (corrected) line widths suggests that they reflect overall correlation times typical of those in the "fast motion" branch of the T_1 curve (Doddrell et al., 1972).

The extent of motional freedom of Met side chains in MBP can be compared, using T_1 values, to motion in side chains of some pentapeptides. For example, if one converts the T_1's (Table I) for S-methylated protein samples to NT_1 values of about 1.5 s (N = number of bound protons; for CH_3, $N = 3$), one finds these to be of the same order of magnitude as those reported for comparable side chain methyl substituents in pentapeptides of the type Gly-Gly-X-Gly-Gly, with X = valine, leucine, and isoleucine (Keim et al., 1973). Similarly, NT_1's found for intact Met S-CH_3 groups in MBP in the present work are 2.2–2.5 s. This compares with NT_1 = 2.9 s for Met S-CH_3 in the pentapeptide Gly-Gly-Met-Gly-Gly (Keim et al., 1974).

One constraint of the methodology employed in this study is the use of a side chain carbon atom as conformational probe to reflect the behavior of the protein backbone (i.e., in folding/unfolding or native/denatured transitions). The usefulness of such a probe must be suspect due to (a) its distance from the backbone (the S-$^{13}CH_3$ is actually a side chain ϵ substituent); and (b) its considerably greater measure of rotational (segmental) freedom as compared with backbone atoms. (The preferable situation, namely, carbon-13 enrichment of individual α-carbon atoms along the protein backbone, can be realized only through biosynthetic and/or semisynthetic methods.) However, a comparison of NT_1's reported for intact myoglobin Met S-CH_3 carbons, 0.42–0.48 s (Jones et al., 1975), suggests that ordered structure (in a protein of molecular weight comparable to MBP) will restrict Met side chain molecular motion, and reduce observed T_1 values. It has also been found that protein side chain methyl groups generally experienced significant increases in T_1's (e.g., Val methyl groups in ribonuclease S) accompanying increased local motion upon protein denaturation (Glushko et al., 1972).

Results for MBP may be contrasted further with the apomyoglobin–myoglobin system, where there are also two Met sites (Met-55 and Met-131 in the 153-residue protein). Jones et al. (1975) observed that apomyoglobin (i.e., the "denatured" form) displayed only one resonance for S-CH_3 (at 167.8 ppm), but the reconstituted (i.e., "native") myoglobin gave two distinct Met S-CH_3 peaks (at 176.77 ppm and 178.15 ppm), attributable to their distinct chemical environments arising from the ordered protein conformation.

Thus, the present results yield a picture of MBP residues freely rotating in solution, although aspects of the present ^{13}C spectra do not preclude the presence of some sort of tertiary structure present in MBP in solution, sufficient to confer a definable overall shape to the molecule, but not so "ordered" as to significantly inhibit molecular motion (as reflected by T_1's and line widths). For instance, we noted a distinct clarification of resonances in the aromatic region with decreasing pH (in accord with the results of Chapman & Moore, 1976), which if not attributable to break-up of aggregation, may suggest pockets of local specific structure.

Interaction of Myelin Basic Protein with Liposomes. Many NMR studies have examined the motional properties of lipids, lipid bilayers, as well as intact biological membranes, including rabbit sciatic nerve (Dea et al., 1972; Williams et al., 1973) and whole myelin itself (Williams & Cordes, 1976). In the latter two instances, protein is present on the order of 25%, but either because natural abundance resonances due to protein cannot be visualized at this level and/or the protein is highly immobilized in the membrane (thus broadening lines below the level of observation) (e.g., Brown & Wüthrich, 1977), it has usually not been possible to observe or study the protein portions of these systems.

In the present work, however, the Met side chain methyl resonances of both methylated and intact bovine myelin basic protein incorporated into PA/PC vesicles (Figure 4) are clearly visible. With the preliminary data of Figure 4, we can only speculate on the origins of spectral effects noted in spectra a–c. Upon addition of protein (Figures 4b–c) broadening of the liposome resonances may signal a rigidifying influence of the protein on the membrane; however, an equally likely explanation for this phenomenon is that addition of protein causes fusion and/or aggregation of liposomes, giving rise to larger particles (vs. free liposomes) whose slower overall reorientation may be manifested by increased line widths. In any case, the influence of the liposome on the overall motion of the protein has resulted in the substantial increase of the line widths of protein (Met methyl group) resonances from ca. 1 Hz in free solution to ca. 15 Hz in the presence of liposomes.[3] For comparison, the model compound benzyloxycarbonyl-*S*-methyl-L-methionine [Z-(*S*-methyl)-L-MetOH] displayed an *S*-methyl resonance whose line width (ca. 4 Hz "uncorrected") was unaffected when a sample was prepared in the presence of liposomes under conditions similar to those in Figure 4.[4] In absolute terms, however, 15 Hz remains considerably below the >100 Hz line widths which arise in highly structured systems (e.g., the collagen triple helix; Torchia et al., 1974). We are presently conducting experiments which may help to delineate further details of a possible membrane/myelin basic protein structural model.

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[3] A preliminary value of T_1 was obtained for the S-$(CH_3)_2$ resonance of methylated bovine MBP incorporated into the PA/PC liposome, using only four spectra with values of t = 10, 100, 500, and 1000 ms. This determination yielded a T_1 = 0.27 s (estimated uncertainty, ±20%). This value corresponds to approximately a 100% decrease vs. corresponding T_1's in free solution and may indicate a decrease in overall and/or segmental motion of S-CH_3 groups of the protein in the presence of the liposomes.

[4] An interesting feature of the Z-(*S*-methyl)-L-MetOH spectrum was the splitting of the two *S*-methyl groups into separate resonances about 1.5 Hz apart, visible also in the presence of liposomes, apparently reflecting their magnetic nonequivalence; this phenomenon was not noted in protein spectra.

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Isolation and Characterization of Lectin Binding Proteins from Murine Lymphoid Cells†

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ABSTRACT: The major cell surface proteins with affinity for the dimeric and tetrameric forms of concanavalin A (Con A), and for lens culinaris hemagglutinin, phytohemagglutinin, the pokeweed mitogens Pa-1, Pa-2, Pa-4, and Pa-5, and for wheat germ agglutinin have been isolated from murine lymphoid cells. These receptor proteins were isolated from radioactively labeled and NP-40 solubilized cell proteins by affinity chromatography using agarose–lectin resins. The specifically bound and eluted lectin binding proteins were analyzed according to their mobilities by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate and in certain cases by immunoprecipitation. Specific depletion of the concanavalin A binding proteins in the NP-40 lysates removed essentially all of the radioactive material which bound to each of the other lectins. The data from this depletion study and from the electrophoresis profiles indicate that each of the mitogens studied share a common set of cell surface glycoprotein acceptors. The nonmitogenic lectin wheat germ agglutinin bound mainly to H-2D and H-2K antigens. The rest of the lectins investigated bound in varying amounts to Ia antigens, surface immunoglobulins, and two T cell associated proteins (tentatively identified as the Thy-1 alloantigen) in addition to the two H-2 antigens. All lectins, except for wheat germ agglutinin, also bound a low molecular weight (20 000) protein in varying amounts. It was shown that antibodies directed against each of the above cell surface glycoproteins compete with Con A for each of these binding sites on intact spleen cells, indicating that these glycoproteins act as lectin acceptors on the cell surface as well as after NP-40 solubilization.

The mechanism of lymphocyte activation has been one of the oldest central problems in cellular immunology. The behavior of lymphocytes and their response to antigens and plant lectins provide a unique opportunity to investigate cellular and molecular events taking place in the induction of cellular differentiation and in expression of the different characteristics. While the recognition of the specific antigen involves only a fraction of the lymphoid cells (i.e., clonal selection), the mitogenicity of certain lectins gives rise to a proliferative response involving a large fraction of the treated cells. It is well known that many of the mitogens specifically produce proliferation

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