CONFORMATION OF MYELIN BASIC PROTEIN IN AQUEOUS SOLUTION FROM NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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SUMMARY : High resolution ^{13}C and ^{1}H NMR spectra of myelin basic protein over a range of pH and concentration indicate that intramolecular folding of the polypeptide chain occurs in the region of residues 85-116. As the pH is raised and the net charge on the protein decreased, intermolecular aggregation occurs between these same regions. The residues 81-118 are invariant in different species and this region is the locus of several chemical specificities of the protein.

INTRODUCTION :

The highly ordered myelin sheaths around axons of peripheral and central nerves present many unsolved problems in their structure and pathology. The myelin is in a dynamic steady state, which can be rendered unstable by agents such as toxins (1), antibodies (2) and certain metal compounds (3). The most important neurological disease, multiple sclerosis, involves a breakdown of myelin in the central nervous system, with consequent serious impairment of function. Of the three important proteins in myelin, attention has been focussed on myelin basic protein (MBP), because it can act as a powerful antigen to produce experimental allergic encephalomyelitis (EAE) a demyelinating disease that has some similarity to multiple sclerosis.

MBP has been purified (4) and sequenced (5,6,7,). Its antigenic properties have been extensively studied (8,9). Although several studies (10-16) on its physicochemical properties have been reported, the conformation of the protein in aqueous solution is still in dispute. Some authors (13,14) favor an ordered, compact prolate ellipsoid model for the structure of the protein, whereas others (10-12, 15,16) tend toward a highly solvated random-coil structure.

Techniques used to study the conformation of the protein have included viscosity, sedimentation velocity, low-angle X-ray scattering, electron microscopy, light scattering, circular dichroism and optical rotatory dispersion. These techniques can yield information only on the gross conformational properties of the protein. Two authors (12,16) have obtained proton nuclear magnetic resonance (NMR) spectra of the protein.

The chemical shift and width of an NMR peak from an individual nucleus are sensitive to the chemical environment around that nucleus. In principle, it should be possible to obtain detailed information about the conformation around a single amino-acid residue in a protein from its ^{1}H NMR spectrum. However, as the chemical shifts of ^{1}H NMR peaks cover a small range (approx. 10 ppm) and the widths of individual proton peaks on a proton spectrum tend to be large (approx. 5-30 Hz), a ^{1}H NMR protein spectrum consists of broad bands of overlapping peaks in which it is difficult if not impossible to resolve peaks from individual protons.

^{13}C NMR chemical shifts cover a wider range (approx. 200 ppm) leading to less overlapping of ^{13}C NMR peaks as compared to ^{1}H NMR peaks. Thus, in principle, more information on individual amino-acid residues in a protein can be obtained from a ^{13}C NMR spectrum than can be obtained from a ^{1}H NMR spectrum. In this communication we present results obtained from ^{13}C and ^{1}H NMR spectra of MBP. These, as far as the authors can ascertain, are the first ^{13}C NMR spectra of MBP to be reported.

EXPERIMENTAL

Myelin basic protein was prepared from bovine central nervous system white matter by the procedure of Eylar et al (4). The use of white matter rather than whole brain reduces the amount of contaminating proteins in the preparation. The protein was purified by passage through a Sephadex G-75 (2.5 x 60 cm) column eluted by pH 2 HCl. Purity of the protein was checked by SDS-polyacrylamide-gel electrophoresis.

The purified protein was dissolved in D_2O or 6M guanidinium

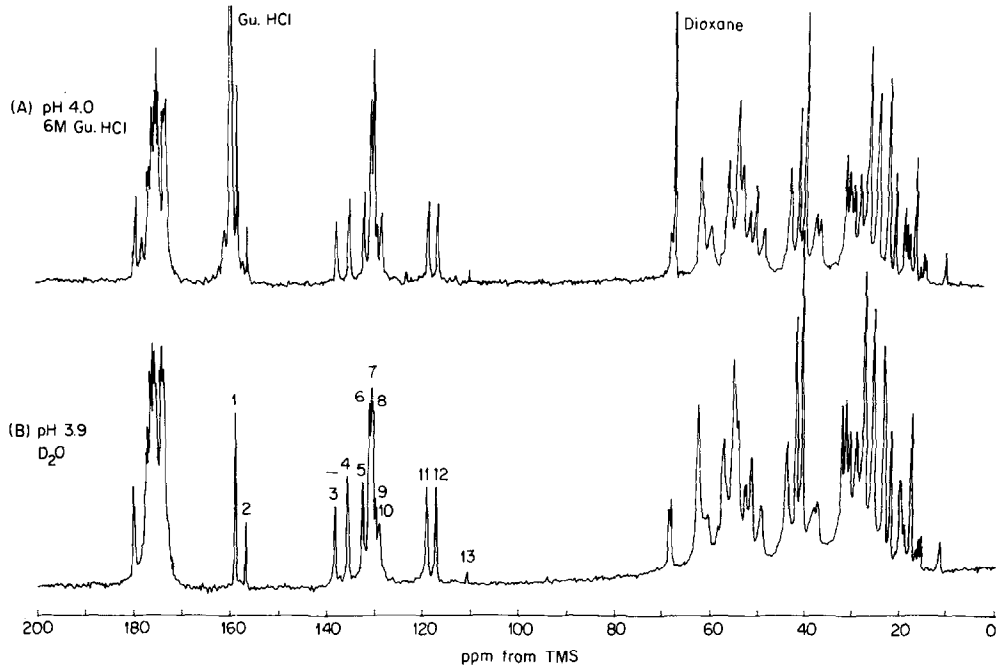

Fig. 1: ^{13}C NMR spectra of myelin basic protein in
(A) 6M guanidinium chloride, pH 4
(B) D_2O, pH 3.9.

hydrochloride (Gu.HCl/D_2O) to give a protein concentration of 200 mg/ml. The pH was adjusted with 6M NaOD and DCl. The pH meter reading was uncorrected for the deuterium isotope effect. ^{13}C NMR spectra were obtained, with 15 mm sample tubes, at 22.625 MHz on a Bruker HX-90 spectrometer by the Fourier transform method. Each spectrum was obtained by averaging approximately 40 000 transients at a 5000 Hz spectral width, 1.5 sec recycle time, 1 Hz digital broadening and 8192 time domain addresses. A 7 μs (45^o) pulse was used to excite the ^{13}C nuclei. Protons were decoupled from the carbon nuclei using a Bruker broadband decoupler. Dioxane was used as an internal standard for measurement of chemical shifts, which are reported in parts per million from external Me_4Si (67.86 ppm upfield from dioxane) (17).

Proton NMR spectra at 270 MHz and a range of concentration from 1 to 20% at pH 3.9 were measured on a Bruker HX-270 spectrometer at the National NMR Centre, Canberra, Australia.

RESULTS AND DISCUSSION

^{13}C NMR spectra of MBP in D_2O and 6M Gu.HCl/D_2O were obtained over a range of pH 3-8.5. Typical ^{13}C NMR spectra of MBP in 6M GuHCl/D_2O, pH 4.0 and D_2O, pH 3.9 are shown in Figs 1A and 1B. At this pH the protein has a net positive charge of 28 and is thought to exist as a monomer (11). The peaks fall into three main groups, arising from carbonyl (165-181 ppm), aromatic (105-145 ppm) and aliphatic (5-70 ppm) carbon atoms.

A common feature of spectra obtained in D_2O solutions is the superposition of sharp resonances onto a broader background. This type of spectrum would be consistent with the protein containing some regions that are structural and others that are capable of free rotation as in a random coil. In 6M Gu.HCl/D_2O solutions the broadened resonances sharpen, which is consistent with the protein being completely a random coil in this solvent. An alternative explanation of the broadening would be a difference in the interaction of solvent and polypeptide chain in D_2O and 6M Gu.HCl/D_2O solutions. This is an unlikely interpretation since the broadened resonances appear to come from amino-acid residues in a localized part of the polypeptide chain.

In the aromatic region the peaks can be identified (17,18,19,20) as arising from (1) the $C\zeta$ of the arginine, (2) the $C\zeta$ of tyrosine, (3) the $C\gamma$ of phenylanine, (4) the $C\varepsilon_1$ of histidine, (5) the $C\delta$ of tyrosine (6) the $C\varepsilon$ of phenylalanine, (7) the $C\delta$ of phenylalanine, (8) the $C\gamma$ of histidine, (9) the $C\gamma$ of tyrosine, (10) the $C\zeta$ of phenylalanine, (11) the $C\delta_2$ of histidine, (12) the $C\varepsilon$ of tyrosine and (13) the $C\gamma$ of the sole tryptophan residue. Of these peaks, the most affected by the addition of Gu.HCl are those arising from phenylalanine residues and from the tryptophan residue, indicating that the tryptophan and some of the

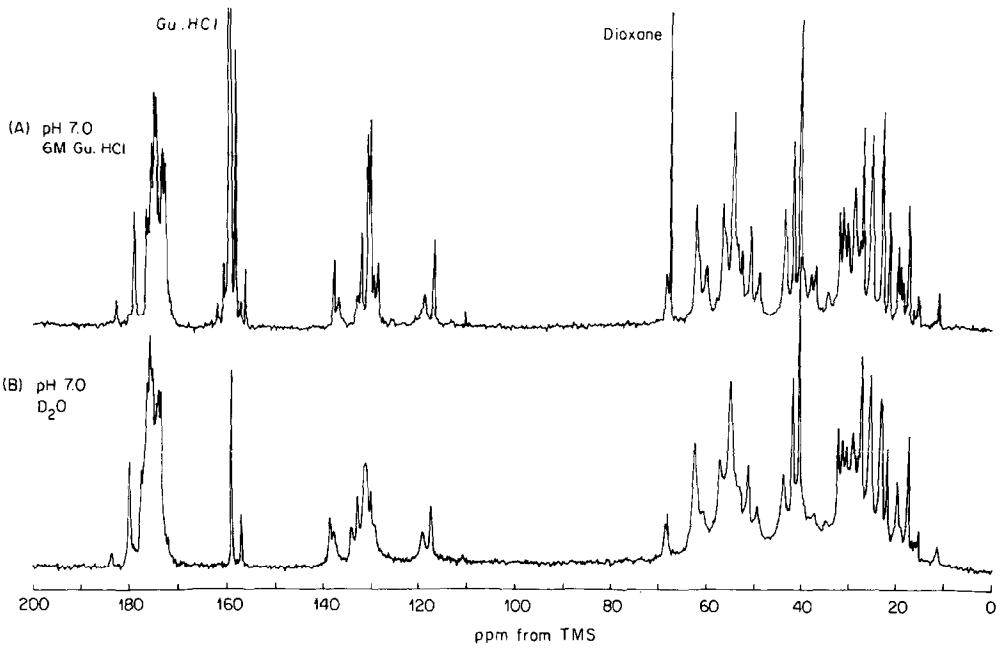

Fig. 2: ^{13}C NMR spectra of myelin basic protein in
(A) 6M guanidinium chloride, pH 7
(B) D_2O, pH 7.

phenylalanine residues are involved in folded regions of the polypeptide chain.

In the aliphatic region the spectrum is more complex and a full assignment of peaks has not been attempted. However, amino-acid residues which have carbons that give rise to peaks in the broadened parts of the spectrum have been identified. From the right these are the isoleucine δ and ε methyl carbons at 11.7 and 16.2 ppm, the valine methyl carbons at 16.9 and 19.3 ppm, the threonine γ methylene at 19.8 ppm and the leucine methyls at 20.4 and 23.6 ppm. At the left of the aliphatic region, broadened peaks at 60, 55, 53 and 33 ppm are composite peaks from a number of amino and α and methylene carbons. By eliminating residues which have carbons that give rise to peaks in only one of the broadened regions we are left with the abovementioned amino-acid residues and proline.

As the pH is raised the protein aggregates. Figs 2A and 2B show spectra of the protein in 6M Gu.HCl/D_2O and D_2O at pH 7. At this point a minimum of 18 charges remain. It can be seen that the spectrum obtained in D_2O at pH 7 is broader than that at pH 3.9 and that the peaks that have broadened the most are those that were already broad at pH 3.9. This result would indicate that the protein is aggregating through structured (folded) regions of the sequence. The broadening of the histidine residues is due to nonequivalence in their pK's and thus chemical shifts as there is little alteration of these peaks when Gu.HCl is added. The glycine Cα at 44 ppm is broadened by aggregation although there was no visible effect at pH 4. The alanine β methyl peak at 18.1 ppm is unaffected by aggregation.

From the amino-acid sequence we can see that the region from residues 85 to 116 comprises a large proportion of the amino-acid residues that give rise to broad peaks. This region contains only one histidine and none of the 14 alanine residues in the sequence, which is consistent with the observation that peaks from these residues are unaffected by folding or aggregation, (although the alanine β methyl tends to be sharp in protein structures irrespective of conformation).

Proton NMR spectra at 270 MHz and a range of concentrations 1 to 20% at pH 3.9 are shown in Fig 3. If the selective broadening in the ^{13}C spectra at pH 4.0 were due to intermolecular folding, we should expect to observe a dependence of peak width on concentrations in the ^{1}H spectra. The spectra in Fig 3 display no dependence of peak width on concentration, in contrast to the strong dependence of peak width on pH in the ^{1}H spectra of MBP reported by Liebes, Zand and Phillips (16), whose results indicate a marked aggregation at higher pH.

We thus conclude that the most consistent interpretation of the NMR spectra is as follows: At low pH the protein in aqueous solution undergoes intramolecular folding in the region of residues 85-116. As their charges are reduced at higher pH, the partly folded polypeptide chains

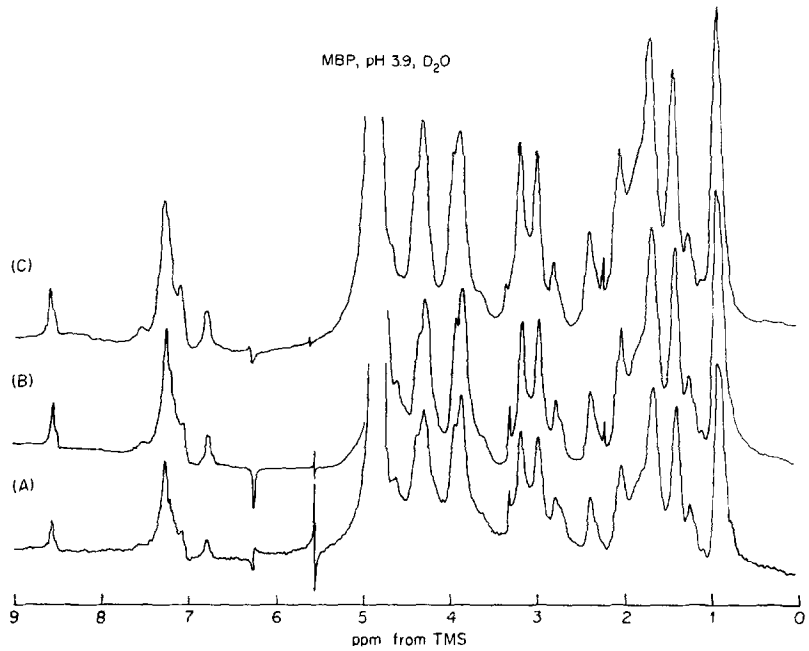

Fig. 3: ^1H NMR spectra of myelin basic protein in D_2O, pH 3.9 at
(A) 1% concentration
(B) 10% concentration
(C) 20% concentration.

undergo intermolecular aggregation through these same folded regions. This behavior is analogous to that suggested for the histones (21).

Residues 81-118 comprise a constant region in amino-acid sequences reported for MBP from man (5), cow (6) and rat (7). This fact suggests the possibility of some highly specialized function, in the structure of the myelin nerve sheath, for this region of the protein. The polymerization of the protein is especially marked in the region 85-116. The only arginine residue in MBP that is methylated by endogenous methylase in the brain is at 107, indicating a highly specific conformation in this region (22). An unusual triplet of prolines occurs at 99-101, and the threonine at 98 is phosphorylated in the native bovine protein (23).

Our study shows that MBP is not simply a random chain polypeptide but rather possesses specialized conformational features (even in aqueous media) in the invariant region. Such conformational features would provide a basis for the specificity of this region as substrate for enzymes. Just as the invariant structures of the histones appear to play a role in their interactions with negatively charged DNA in chromatin superstructures (21), so may the analogous properties of MBP be essential for its interactions with negatively charged lipids in the myelin superstructure. The ordered three dimensional structure of the myelin sheath is determined to a considerable extent by the balance of electrostatic and dispersion forces between the surfaces of the lamellar membranes (24). We would suggest that the role of MBP is to stabilize the helical array of myelin lamellae relative to either the vesicular aggregates or the unwound lamellae seen in demyelinative disorders.

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