N.m.r. studies of myelin basic protein

Conformation of a peptide that is an antigenic determinant for B-cell reactivity

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The peptide Gly-Arg-Ala-Ser-Asp-Tyr-Lys-Ser, derived from myelin basic protein (MBP), is part of an epitope to monoclonal antibodies to human MBP. Its conformation has been studied in aqueous solution by high-resolution one- and two-dimensional ^1H and ^{13}C n.m.r. Two-dimensional correlated spectroscopy, pH titrations and one-dimensional spin-decoupling techniques were employed to assign the spectra observed from both nuclei. Amide proton temperature coefficients, coupling constants, ^{13}C spin-lattice relaxation times and nuclear-Overhauser-effect data provide evidence that the solution conformations of the octapeptide include a type-II β -turn with a hydrogen bond between the CO group of Arg² and the NH group of Asp⁵. The results are discussed in view of a possible conformation of the antibody receptor site.

The structural and conformational basis for the immunological properties of myelin basic protein (MBP) has been investigated in a series of studies using ^1H and ^{13}C n.m.r. and c.d., of the protein and peptides derived from it (Mendz *et al.*, 1982, 1984; Mendz & Moore, 1983; Nygaard *et al.*, 1984). The present work considers an antigenic determinant of the human protein that reacts with monoclonal immunoglobulins.

Experimental autoimmune encephalomyelitis is a T-cell-mediated demyelinating disease of the central nervous system that can be induced in many animal species by subcutaneous injection of MBP in Freund's complete adjuvant. In some species the disease can be prevented or cured by injection of MBP without FCA at appropriate times respectively before or after the challenge with MBP + FCA (Cuzner & Davison, 1979). Co-operative interaction between B- and T-lymphocytes appears to be necessary for the induction of experimental autoimmune encephalomyelitis (Willenborg & Prowse, 1983).

Abbreviations used: c.o.s.y., correlated n.m.r. spectroscopy; DSS, 4,4-dimethyl-4-silapentane-1-sulphonate; FCA, Freund's complete adjuvant; (b-, h- and p-) MBP, (bovine, human and porcine) myelin basic protein; n.o.e.d., nuclear Overhauser difference spectra; n.o.e.s.y., nuclear-Overhauser-enhancement spectroscopy; s.d.d., spin decoupling difference; t.o.e.d., truncated n.o.e.d.; 1D and 2D, one- and two-dimensional.

The fact that multiple sclerosis is in several respects analogous to a chronic progressive form of experimental autoimmune encephalomyelitis provided the rationale for a trial of p-MBP in the treatment of multiple sclerosis, the results of which were disappointing (Romine & Salk, 1983).

Three laboratories have prepared monoclonal antibodies to h-MBP (Sires *et al.*, 1981; Carnegie *et al.*, 1983; Fritz & Chou, 1983). Two of these antibodies react with an epitope consisting of a linear sequence of amino acid residues comprising the region 130–140 of the protein. b-MBP, but not p-MBP, also reacts with these monoclonal antibodies. The antigenic determinant that reacts with the monoclonal immunoglobulins includes the linear sequence Gly-Arg-Ala-Ser-Asp-Tyr-Lys-Ser-Ala-His in both human and bovine proteins. In p-MBP, which is non-reactive, the two serine residues in this sequence have been replaced by proline residues. A ^1H n.m.r. study of the interaction of b-MBP with one of the monoclonal antibodies showed a strong effect at the tyrosine residue (Tyr-135) in the basic protein, but no effect at the histidine residue (His-139) (Mendz *et al.*, 1985). A preliminary n.m.r. experiment with the synthetic octapeptide, Gly-Arg-Ala-Ser-Asp-Tyr-Lys-Ser, binding to the Fab fragment of the monoclonal antibody, gave evidence for two different slowly exchanging conformations of the peptide in the presence of the Fab. To understand the interaction of the epitopic peptide with the

antibody, it is necessary first to delineate the conformation of the peptide itself in aqueous solution; such an analysis is the aim of the present study.

Materials and methods

The octapeptide was synthesized by Peninsula Laboratories, San Carlos, CA, U.S.A. Analysis by liquid chromatography showed no detectable impurities. Before n.m.r. measurements on non-exchangeable protons the peptide was freeze-dried once from 99.5% $^2\text{H}_2\text{O}$ and once from '100%' $^2\text{H}_2\text{O}$ (Merck, Sharp and Dohme, Point Claire, Quebec, Canada). Measurements of amide resonances were made after freeze-drying and solution in doubly distilled $^1\text{H}_2\text{O}/^2\text{H}_2\text{O}$ (9:1, v/v) mixtures. The pH of solutions was adjusted with NaOH or HCl as required, and measured with a combined Ingold 6030-02 microelectrode. Cited values are meter readings. The ^1H chemical shifts are referred to an internal standard of DSS at zero p.p.m. (Menz et al., 1982).

Chemical shifts and linewidths of the amide-proton resonances did not change appreciably as the concentration of peptide was varied from 1.0 to 50 mM. The only concentration effect was a loss in intensity of the most downfield amide-proton resonances, which may be due to autocatalysis of the exchange reaction.

Temperature coefficients of the amide resonances were measured from 283 to 333 K at a concentration of 3.0 mM and pH 4.0. The temperature was calibrated by the method of Kaplan et al. (1975).

Spectra were obtained with a Bruker WM-400 n.m.r. spectrometer operating in the Fourier-transform mode with quadrature detection. For one-dimensional (1D) proton spectra, a 90° (7–8 μs) pulse, spectral width 4800 Hz, acquisition time 1.7 s and 128 scans were typically used. Spin-decoupling difference spectra were acquired by the procedure of Brown & Wüthrich (1981). 1D n.o.e.d. were obtained with a saturation time of 2.0 s by the method of Jones et al. (1978) and t.o.e.d. by the method of Braun et al. (1981) with saturation times from 0.04 to 1.3 s. These 1D n.o.e. measurements were used primarily to check results of 2D n.o.e. experiments. The ^1H 2D techniques employed were c.o.s.y. and n.o.e.s.y., with the parameters as previously described (Nygaard et al., 1984), except for a fixed mixing time of 0.5 s; these 2D experiments were made with 35 mM solutions, pH 5.5 at 298 K.

A heteronuclear shift-correlated 2D spectrum with standard Bruker programs (Bain et al., 1981) allowed the ^{13}C resonances of protonated carbon nuclei to be assigned from the ^1H assignments. A

70 mM solution was used at 305 K and pH 5.5. There were 2000 data points per scan and 256 experiments were transformed with no window functions. A 1D ^{13}C spectrum was obtained under the same conditions with 128 scans and repetition time 5.5 s.

The longitudinal relaxation times, T_1 , of the ^{13}C nuclei were measured by the inversion-recovery method (Vold et al., 1968). The 180° pulse width was 44 μs , and 16 experiments were run with delay times between 0.001 and 5.5 s. The T_1 values were calculated by a non-linear regression program (Margetson & Moore, 1980). The ^{13}C chemical shifts were obtained with respect to an internal standard of dioxan at 67.86 p.p.m. (Oldfield et al., 1975). Heteronuclear ^{13}C – ^1H n.o.e. measurements were made by the gated-decoupling technique (Freeman et al., 1972). Samples were prepared in 3 mM-EDTA sodium salt solutions to suppress the effects of paramagnetic metal ions. Oxygen was removed by purging with high-purity nitrogen.

Results

^1H assignments

The first step in any n.m.r. conformational study is the assignment of the spectral lines. This was accomplished for all the detectable protons through pH titrations, spin-decoupling difference (single or double decoupling: s.d.d. or d.s.d.d.), and the 2D c.o.s.y. spectrum of the peptide in water solution. A 1D proton spectrum is shown in Fig. 1. The chemical shifts of resonances, δ , are included in Table 1. The individual assignments are summarized as follows.

Gly¹. On the scalar coupling map the only connectivity of this group is to a resonance in the amide region. The absence of other cross-peaks indicates an assignment to glycine methylene protons.

Arg². This characteristic spin system displays a unique network of connectivities in the c.o.s.y. contour plot (Fig. 2). Arg- δ is identified by its connection with Arg- ϵ in the amide region. The connectivities in the scalar correlated map then give the positions of the γ -, β - and α -resonances. Accurate chemical shift values for Arg- γ and Arg- β are obtained by s.d.d. and d.s.d.d. Irradiation of Arg- α and one of the Arg- γ resonances gives a pattern for Arg- β . Irradiation of Arg- α and the other Arg- γ gives a slightly different pattern. Similarly d.s.d.d. of Arg- δ and Arg- β gives peaks for Arg- γ .

Ala³. These resonances are identified by the unique connection of the only upfield methyl moiety in the c.o.s.y. map with the resonance corresponding to the methine proton (Fig. 2).

Ser⁴ and Ser⁸. The residue type is readily

Table 1. ^1H Chemical shifts of resonances, $^3J_{\text{NH}-\alpha\text{-CH}}$ coupling constants and (ϕ) torsional angles at 298 K and pH 4.0

Residue	Proton	δ (p.p.m.)	$^3J_{\text{NH}-\alpha\text{-CH}}$ (Hz)	$\phi \pm 4$ ($^\circ$)
Gly ¹	$\alpha\text{-CH}_2$	3.943, 3.943		
Arg ²	$\alpha\text{-NH}$	8.202	8.22	−138.0
	$\alpha\text{-CH}$	4.441		−102.0
	$\beta\text{-CH}_2$	1.803, 1.769		
	$\gamma\text{-CH}_2$	1.760, 1.708		
	$\delta\text{-CH}_2$	3.243, 3.226		
	$\varepsilon\text{-NH}$	7.276		
Ala ³	$\alpha\text{-NH}$	8.734	5.28	−170.00
	$\alpha\text{-CH}$	4.410		−70.00
	$\beta\text{-CH}_3$	1.539		32.00 88.00
Ser ⁴	$\alpha\text{-NH}$	8.388	6.87	−164.00 −76.00
	$\alpha\text{-CH}$	4.443		42.00
	$\beta\text{-CH}_2$	4.001, 3.902		78.00
Asp ⁵	$\alpha\text{-NH}$	8.296	7.04	−153.0
	$\alpha\text{-CH}$	4.666		−87.0
	$\beta\text{-CH}_2$	2.705, 2.690		
Tyr ⁶	$\alpha\text{-NH}$	8.057	7.04	−153.0
	$\alpha\text{-CH}$	4.611		−87.0
	$\beta\text{-CH}_2$	3.145, 3.083		
	$\delta_1\text{-}, \delta_2\text{-CH}$	7.217, 7.217		
	$\varepsilon_1\text{-}, \varepsilon_2\text{-CH}$	6.918, 6.918		
Lys ⁷	$\alpha\text{-NH}$	8.734	7.04	−153.0
	$\alpha\text{-CH}$	4.441		−87.0
	$\beta\text{-CH}_2$	1.902, 1.802		
	$\gamma\text{-CH}_2$	1.457, 1.457		
	$\delta\text{-CH}_2$	1.749, 1.749		
	$\varepsilon\text{-CH}_2$	3.086, 3.074		
Ser ⁸	$\alpha\text{-NH}$	8.001	7.04	−153.0
	$\alpha\text{-CH}$	4.317		−87.0
	$\beta\text{-CH}_2$	3.983, 3.983		

identified by the special connection of a methine proton to a markedly downfield $\beta\text{-CH}_2$ group. One residue has equivalent β -protons and the other a characteristic (2×4) AMX pattern (Fig. 1). A pH titration distinguishes the C-terminal Ser⁸ resonances from those of Ser⁴. Between pH 2.05 and 4.70, changes in chemical shifts in p.p.m. are Ser⁴-NH, 0.087; Ser⁸-NH, 0.157; Ser⁴- α , 0.030; Ser⁸- α , 0.196. Thus the methylene protons of Ser⁸ are equivalent, whereas the Ser⁴ β -methylene protons are non-equivalent.

Asp⁵ and Tyr⁶. These two residue types have similar patterns of $\alpha\text{-CH}-\beta\text{-CH}_2$ connectivities (Fig. 2); the β -proton resonances are located between 2.5 and 3.3 p.p.m. and display a typical (2×4) line pattern and no other connections. Between pH 2.05 and 4.70 one group of lines shifts upfield by 0.232 p.p.m. and the other shifts downfield by 0.010 p.p.m. The former is assigned to Asp- β and the latter to Tyr- β . Assignment of the aromatic tyrosine resonances is obvious on comparing solutions in $^1\text{H}_2\text{O}$ and $^2\text{H}_2\text{O}$.

Lys⁷. Resonances are identified by a unique pattern of connections in the c.o.s.y. map, starting at the Lys- ϵ resonances and including the most upfield peaks of the Lys- γ protons. Experiments by d.s.d.d. show the $\gamma\text{-CH}_2$ are equivalent as are the $\delta\text{-CH}_2$ protons. The $\alpha\text{-NH}$ peak, which partially disappears and overlaps the $\alpha\text{-NH}$ resonance of Ala³ at 298 K, is assigned by s.d.d. at 283 K, where it is well resolved from the Ala³- $\alpha\text{-NH}$ and displays no loss in intensity due to autocatalytic exchange reactions. (The experiments were done with 3.0 mM solutions.)

^{13}C assignments

The shift-correlated 2D contour map in Fig. 3 leads to assignments of ^{13}C resonances. Chemical-shift data from a 1D spectrum are given in Table 2. The Arg²- $\beta\text{-CH}_2$ resonance is missing in the 2D spectrum; it was assigned in 1D spectrum by default, with a chemical shift in good agreement with that observed in MBP itself (Chapman & Moore, 1978). Resonances of Tyr- γ , Tyr- ζ , and

Fig. 2. 2D correlated contour map (c.o.s.y.) at pH 5.5, 298 K and 35 mM

The resonances along the diagonal can be identified by reference to Fig. 1. ----, C α H-N α H peaks; - - - - -, C α H-C β H peaks; —, other explicitly indicated connectivities. ω_1 is the frequency corresponding to the evolution time and ω_2 is the frequency corresponding to the detection time.

Arg- ζ were assigned by comparison with concordant values in MBP.

Amide-proton temperature coefficients

Linear least-squares regression analysis of the variations with temperature of the chemical shifts of the NH amide-proton resonances gave the $-d\delta/dT$ values in Table 3. As a control of the $d\delta/dT$ of the amide protons, the δ of the α -CH protons were practically independent of temperature (Higashima *et al.*, 1979). The values of the coefficients are increased in urea solutions, and those resonances that initially display lower temperature coefficients experience greater increases.

T_1 and heteronuclear n.o.e. values for ^{13}C nuclei

The NT_1 (spin-lattice relaxation times) and n.o.e. values for ^{13}C nuclei in the peptide are recorded in Table 2. Measured n.o.e. values indicate that all T_1 values cited are determined

solely by the dipolar mechanism (Doddrell *et al.*, 1972). The structural interest in NT_1 is the information provided regarding relative mobilities of different regions of the peptide backbone and side chains. Applications of the NT_1 equation for a rigid rotor (Doddrell *et al.*, 1972) to the Tyr 6 - α -CH T_1 of 0.338 s gives a correlation time for the overall isotropic motion of the peptide chain, τ_{mol} , of 0.16 ns, based on a C-H bond length of 109 pm. Side-chain NT_1 values were analysed by the method of Wallach (1967) and Levine *et al.* (1974). This gives correlation times, τ_{int} , for rotations in side-chain carbon-carbon bonds. Although the absolute values of τ_{int} are dependent on the approximation made in the dynamic model, the values for different positions along the backbone or along side chains provide information concerning relative mobilities. In particular, the NT_1 of Asp 5 - and Tyr 6 - α -CH are shorter than those of other α -CH carbon atoms, suggesting relatively restricted mobility in this region of the backbone.

Fig. 3. Aliphatic region of the 2D ^{13}C - ^1H heteronuclear shift correlated contour map at pH 5.5, 305 K and 70 mM. The corresponding 1D proton (left-hand side) and carbon-13 (top) spectra are also shown. δ_{H} is the chemical shift of proton nuclei in ^1H spectrum along ω_1 , and δ_{C} is the chemical shift of carbon nuclei in ^{13}C spectrum along ω_2 .

The NT_1 data also show that the side-chain carbon atoms of Arg² are considerably less mobile than those of Lys⁷, suggesting some restriction on freedom of motion in this *N*-terminal region of the peptide.

Coupling constants

The $^3J_{\alpha\text{-NH}-\alpha\text{-CH}}$ coupling constants derived from the fine structure of resonances are listed in Table 1. The torsional angles, ϕ , in Table 1 were calculated from the Karplus relations and the measured 3J values. The parameters used are based on data for the ferrichromes; the solid-state structures of these rigid peptides are believed not to change on solution in water (De Marco *et al.*, 1978). The ϕ values for the MBP peptide, however, are likely to be weighted averages over more than one conformation.

Homonuclear ^1H nuclear-Overhauser effects

N.o.e. data are particularly useful for conformational studies, since the dipolar interaction is strongly dependent on internuclear distance. Thus the occurrence of an n.o.e. between protons on non-adjacent residues provides an immediate indication of a compact conformation of high statistical weight. Because of the small size of this peptide, and its small apparent correlation time

(~ 0.2 ns), the observable n.o.e. at 400 MHz are restricted to small values. They were detected in a 2D n.o.e.s.y. experiment, the result of which is shown in Fig. 4. Only two long-range n.o.e. are marked on the contour plot. Other observable n.o.e. are either intra-residue or between protons on nearest-neighbour residues. The 2D n.o.e. were confirmed by 1D experiments, mainly n.o.e.d. but also t.o.e.d., and the 1D experiments revealed, in addition, two other long-range n.o.e., namely Arg²- δ to Tyr⁶- ϵ and Tyr⁶- ϵ to Ser⁸- α .

It was not practical to obtain n.o.e. for the amide protons in H_2O solution. At low concentrations the water peak imposes problems of dynamic range, and at high concentrations the downfield amide resonances disappear, probably as a consequence of autocatalytic exchange effects.

Discussion

As the n.m.r. data were obtained, it became evident that the octapeptide possesses unusually compact structures for a linear peptide in aqueous solution. Temperature coefficients of amide-proton chemical shifts, $-d\delta/dT$, have been a useful indication of relative accessibility of these protons to solvent (Kopple *et al.*, 1963). The low values of the temperature coefficients and the longitudinal

Table 2. ^{13}C Chemical shifts of resonances, spin-lattice relaxation times (NT_1) and nuclear Overhauser enhancements for proton-bearing carbon nuclei

Rotational correlation times for internal motion (τ_{int}) were calculated by assuming isotropic overall motion of the peptide chain with $\tau_{\text{mol}} = 0.16\text{ ns}$.

Residue	Carbon	δ (p.p.m.)	NT_1 (ms)	N.o.e.	τ_{int} (ns)
Gly ¹	$\alpha\text{-CH}_2$	41.710	736	1.51	0.049
Arg ²	$\alpha\text{-CH}$	54.553	394	1.93	0.331
	$\beta\text{-CH}_2$	29.702	544	1.98	0.093
	$\gamma\text{-CH}_2$	25.439	840	1.88	0.039
	$\delta\text{-CH}_2$	41.807	842	2.05	0.039
	$\zeta\text{-C}$	158.018			
Ala ³	$\alpha\text{-CH}$	51.363	442	1.71	0.181
	$\beta\text{-CH}_3$	17.602	2064	1.88	0.016
Ser ⁴	$\alpha\text{-CH}$	56.944	392	1.69	0.344
	$\beta\text{-CH}_2$	62.093	498	1.86	0.119
Asp ⁵	$\alpha\text{-CH}$	52.818	374	1.77	0.513
	$\beta\text{-CH}_2$	39.594	394	2.34	0.331
Tyr ⁶	$\alpha\text{-CH}$	56.414	338	1.48	—
	$\beta\text{-CH}_2$	37.156	374	1.89	0.542
	$\gamma\text{-C}$	129.237	1658	1.00	
	$\delta_1\text{-}, \delta_2\text{-CH}$	131.745	429	1.69	0.206
	$\epsilon_1\text{-}, \epsilon_2\text{-CH}$	116.758	454	1.73	0.163
	$\zeta\text{-C}$	155.677			
Lys ⁷	$\alpha\text{-CH}$	54.553	394	1.93	0.331
	$\beta\text{-CH}_2$	31.748	516	1.86	0.108
	$\gamma\text{-CH}_2$	22.995	932	1.86	0.033
	$\delta\text{-CH}_2$	27.360	1516	1.86	0.017
	$\epsilon\text{-CH}_2$	40.525	2278	2.18	0.010
Ser ⁸	$\alpha\text{-CH}$	53.382	568	2.72	0.084
	$\beta\text{-CH}_2$	63.362	692	1.99	0.055

Table 3. Temperature coefficients of amide-proton resonances in urea solutions

Residue	[Urea] (M) ...	−dδ/dT (p.p.b/K)			Increase between 0 and 12M-urea (%)
		0	6	12	
Gly _α ¹	—	—	—	—	
Arg _α ²	5.14	6.02	6.37		19
Ala _α ³	8.25	8.43	8.55		3
Ser _α ⁴	7.45	7.52	7.64		2
Asp _α ⁵	3.92	5.03	5.41		28
Tyr _α ⁶	3.66	4.71	4.98		26
Lys _α ⁷	4.79	—	—		
Ser _α ⁸	5.37	6.32	6.62		19
Arg _ε ²	1.70	2.09	2.21		23

relaxation times of the amide protons and methine carbon nuclei respectively, of residues Asp⁵ and Tyr⁶ indicate that these NH groups are protected from solvent and that the backbones of the residues are restricted in mobility. The backbone of residue Arg² also is unusually restricted compared, for example, with that of residues Ala³ and Ser⁴. In flexible linear peptides, the spin-lattice relaxation times of the methine carbon nuclei are usually very similar, except for those of the nuclei at the ends of the chain, which have

notably larger mobilities. A substantial increase in mobility is also usually found in the methylene carbon nuclei of long aliphatic side chains on proceeding outward from the main chain. Such an effect is evident in the side-chain carbon nuclei of Lys⁷. The data for Arg², therefore, are anomalous and suggest that its side chain is restricted in mobility.

When the peptide is dissolved in aq. 6M- or 12M-urea solution, the lower $-\text{d}\delta/\text{d}T$ values are disproportionately raised, so that differences

Fig. 4. 2D dipolar coupling-contour map (n.o.e.s.y.) at pH 5.5, 298 K and 35 mm. Two long-range inter-residue n.o.e.s are indicated.

between different α -NH groups become less marked. This effect suggests a breaking of special structures, such as hydrogen bonds, in the urea solutions.

The n.o.e. between protons of non-adjacent residues (Ala³- β to Lys⁷- ϵ ; Arg²- α to Lys⁷- ϵ ; Arg²- δ to Tyr⁶- ϵ ; Tyr⁶- ϵ to Ser⁸- α) can be explained only by non-extended structures for the peptide. It is unlikely that all close interproton distances in the peptide have yielded observable n.o.e. at 400 MHz; nevertheless, the n.o.e. that have been measured provide constraints on the delineation of permissible structures.

From the 3J values for Ala³ and Ser⁴ peptide bonds, four torsional angles can be calculated for each residue. Of the sterically possible pairs of angles, two, (-70° , -76°) and (-70° , 78°), are close to those corresponding to β -turns, type I and II respectively. The theoretical values are -60° , -90° for type I and -60° , 80° for type II. The

temperature coefficient of Ser⁴-NH indicates that this proton is exposed, making the latter type of bond more likely.

The n.m.r. data suggest the existence of conformations of high statistical weight that include a type-II β -turn with a hydrogen bond between the CO of Arg² and the NH of Asp⁵. A model of one such structure is shown in Fig. 5. The low mobility of the Arg² side chain is explained by the restriction due to hydrogen-bonding of the backbone at the Arg² residue. The observed Arg²- δ to Tyr⁶- ϵ n.o.e. leads to the side-chain appositions shown in the model.

The Lys⁷ side chain, which is more mobile than that of Arg², sometimes approaches the Ala³ methyl group (Ala³- β to Lys⁷- ϵ n.o.e.); this is the structure shown in the model of Fig. 5. At other times, Lys⁷ gets closer to Arg²; this structure can be derived from the one depicted in the model simply by shifting the Lys⁷ side chain. Arg² is

Fig. 5. (a) Model of a conformation of high statistical weight consistent with the n.m.r. data for the peptide and (b) the same model rotated 180° about a vertical axis and 90° about a horizontal axis, both axes on the plane of the paper

The arrows indicate the position of the CO...HN hydrogen bond. The single-letter codes are the same as in Fig. 1.

fairly boxed-in by Gly¹, Tyr⁶ and Lys⁷, and to allow some room the Tyr⁶ ring is tilted toward Ser⁸ (Tyr⁶- ϵ to Ser⁸- α n.o.e.).

The conformation of the octapeptide, as revealed by this n.m.r. study in aqueous solution, may be compared with the interaction of the epitopic region of MBP with the monoclonal antibody (Mendz *et al.*, 1985). The position of the tyrosine residue is consistent with its important role in binding to the receptor site. The antibody-antigen binding involves an electrostatic interaction with at least one of the three charged side chains in the epitope. The Asp⁵ residue is definitely the least available for such receptor binding. A decision between the positively charged Arg² and Lys⁷ side chains cannot as yet be made, but the position of the Arg² positive charge adjacent to the aromatic ring of Tyr⁶ provides a conformation reminiscent of a two-pronged electrical plug. It will be of interest to determine whether the conformation of the receptor site of the antibody will reveal a complementary molecular socket for such a plug.

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