BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **224,** 5–9 (1996)
ARTICLE NO. 0975

Conformation of an Antigenic Determinant for Experimental Autoimmune Neuritis

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Received May 22, 1996

The conformation of SP-26, the synthetic peptide (residues 53–78) of myelin P2 protein that causes experimental autoimmune neuritis (EAN) in the peripheral nervous system, has been investigated in D_2O using Fourier transform infra-red spectroscopy. Turns were found in 26% of the residues in the peptide, with rest of the residues in random coil (72%). The presence of 26% turns agrees well with the number of residues forming three turns in the antigenic region of the intact protein and the number of turns correlates well with the severity of EAN. Since turns also exist in peptides inducing experimental autoimmune encephalomyelitis, the central nervous system counterpart of EAN, turn structure may be a common structural motif for these closely related autoimmune neurological disorders.

P2 protein is one of the major proteins found in peripheral nervous system (PNS) myelin and has been identified as an antigen responsible for induction of experimental autoimmune neuritis (EAN)(1-3). EAN is an inflammatory demyelinating disease of the PNS and is considered to be an appropriate model for a human demyelinating disease, the Guillain-Barre syndrome. P2 protein is also believed to have a structural role in the myelin sheath. The antigenic determinant of P2 protein has been located within the region defined by residues 53-78 (4,5).

The conformation of P2 protein in aqueous solution has been studied by our group using circular dichroism (CD), nuclear magnetic resonance (NMR) spectroscopy and Fourier transform infra-red (FTIR) spectroscopy (24,25). Each of these studies showed that P2 protein consists largely of β-structure with a small amount of α-helix and turns. CN1 peptide, comprising residues 21-113, has also been studied using CD, NMR and FTIR, showing that the peptide is largely random with turns as a major secondary structural component (24,26).

According to the x-ray crystal structure of P2 protein, the antigenic region 53-78 contains three turns formed by residues 56, 57, 66-68, and 75, 76 (6). Turns commonly exist in peptide epitopes inducing experimental autoimmune encephalomyelitis (EAE), the central nervous system counterpart of EAN (7-10). To examine whether the turns found in the intact P2 protein still exist in the peptide fragment containing the antigenic determinant and to investigate the relationship between the conformation and the antigenic activity, we have synthesized SP-26, corresponding to amino acid residues 53-78 of P2 protein and the conformation was studied using FTIR. In this report, we present evidence that turn conformation is a major secondary structural component found in the antigenic peptide 53-78 of myelin P2 protein.

MATERIALS AND METHODS

Peptide synthesis. SP-26 (*see* Fig. 3 for the sequence) was synthesized by the Fmoc polyamide solid-phase synthesis procedure and purified as described previously (11).

Fourier transform infrared spectroscopy. IR spectra were measured using a Digilab FTS-20/80 spectrometer

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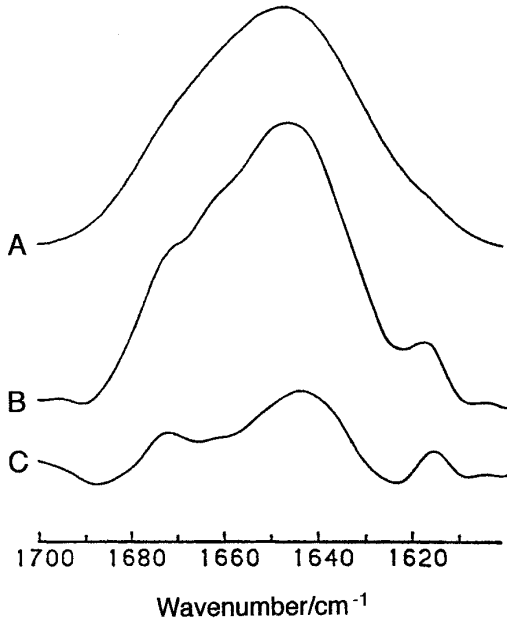

FIG. 1. Infrared spectra in the amide I region of SP-26 in D_2O: (A) original spectrum; (B) deconvolved spectrum; (C) derivative spectrum.

equipped with a liquid nitrogen-cooled mercury cadmium telluride (MCT) detector. 1000 scans were averaged, apodized with a triangular function, and Fourier transformed to give a nominal resolution of 2 cm^{-1}. Solution spectra were recorded in a CaF_2 cell (pathlength 0.050 mm) in the single beam mode by automatic subtraction of the air spectrum. The peptide spectra were then calculated by subtraction of the pure solvent spectra. The spectra were then resolution-enhanced by using Fourier self-deconvolution (12) and second order derivation (13). The spectra were deconvolved using a half-width Lorentzian line of 20 cm^{-1} and a k value of 2.0. The derivative spectra were calculated using a power of 2 and a passband edge of 0.3. The number of component bands and approximate estimates of the wavenumbers of these bands were obtained from both the resulting deconvolved spectra and the second derivative of the original spectrum.

Computer analysis. To obtain the fractions of the various secondary components, a curve-fitting FORTRAN program was used, based on a Gauss-Newton least-squares minimization procedure (14). Gaussian band shapes were assumed in the program and the relative areas of the component bands gave the required fractions (15). Iteration was continued to convergence at a low root mean square error (~0.001%). Curve-fitting procedures, based on a Gauss-Newton least-squares method, were then applied to get the relative areas of the component bands.

RESULTS AND DISCUSSION

FTIR spectroscopy has developed in recent years as an effective technique for the examination of protein and peptide conformations (16). The amide I mode of proteins and peptides, due mainly to C=O stretching, has proved to be the most useful, since this broad mode consists of overlapping components which can be assigned to specific secondary structures. In addition, improvements in instrumentation and analytical methods have allowed accurate quantitative results to be obtained.

Figure 1 shows the amide I region of SP-26 at 20°C in D_2O along with the resolution-enhanced bands. The spectrum (Fig. 1.A) shows a maximum at about 1648 cm^{-1} and a broad shoulder between 1660 and 1680 cm^{-1}. Overall the spectrum is not symmetrical, indicating that the amide I band is a composite of multiple components. The component bands can be easily visualized by applying computational procedures of resolution enhancement (12,13). The spectrum of the SP-26 in D_2O after Fourier deconvolution is shown in Fig. 1.B. The same

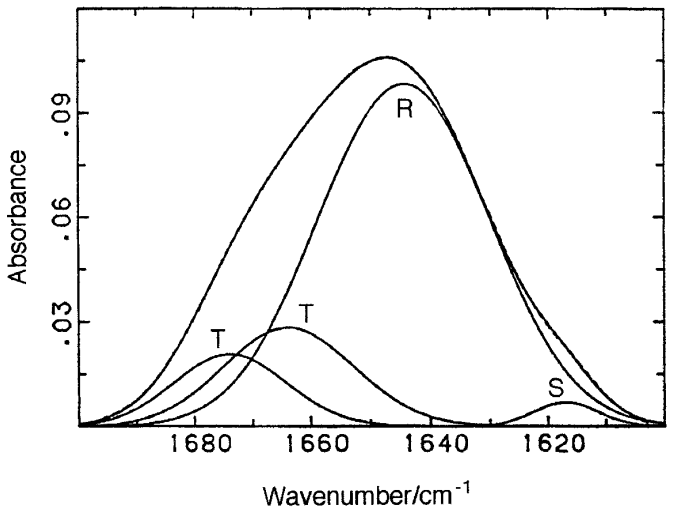

FIG. 2. The amide I region of the original spectrum with the best-fitted individual component bands for SP-26 in D_2O. The symbols T, R, S represent turns, random coil and side-chain contributions, respectively. The dashed line which completely overlaps with the original spectrum represents the calculated spectrum.

spectrum after Fourier derivation is shown in Fig. 1.C. Application of either of the two resolution enhancement procedures reveals the presence of four component bands in the amide I region. The frequencies of the component bands identified in the resolution-enhanced spectra are used subsequently as input parameters for curve-fitting and are shown in Fig. 2, where the component bands are drawn together with the original contour. The assignments of these bands to particular types of secondary structure were based on a study by Byler and Susi (15) of 21 globular proteins in D2O and are given in Table 1. The most prominent feature in the amide I region of the infrared spectrum of SP-26 is the strong band at 1644 cm^{-1}, which is characteristic of random coil structure. Besides the main band at 1644 cm^{-1}, the only other significant contributions to the amide I region - bands at 1664 and 1674 cm^{-1}- were attributed to turns. The component band at 1674 cm^{-1} can be assigned as either turn or β-structure, but without one or two accompanying lower component bands at around 1630 cm^{-1} the band at 1674 cm^{-1} can only be assigned to turn. There is also a small mode at 1617 cm^{-1} which may be assigned to the side-chain vibrations (17). The above result indicates that the peptide adopts about 26% turns, with the remainder in the form of random coil.

The present FT-IR study on the antigenic peptide SP-26 shows that the peptide exists mainly as a random coil with about 26% turns in aqueous solution. The presence of 26% turns corresponds to roughyl 7 residues, which agrees well with the number of residues forming three turns in this region of the protein structure. In the crystal structure, residues 56, 57, 66-

TABLE 1
Results of Secondary Structure Analysis of the Amide I Band of SP-26 in D_2O

Wavenumber [cm^{-}]	area[%]	type
1674	10	turn
1664	16	turn
1644	72	random
1617	2	side chain

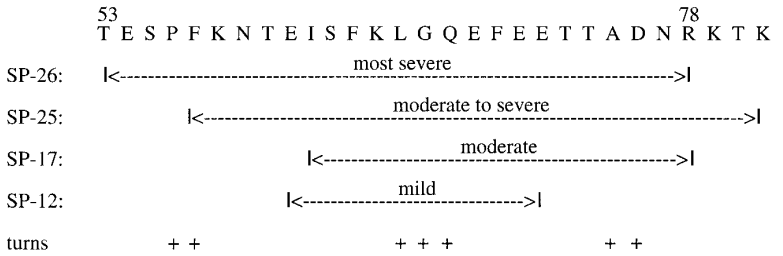

FIG. 3. Alignment of four peptides that have been reported to induce both clinical and histological EAN with the sequence of the P2 protein. The clinical severity of the disease was compared with the number and position of turns.

68, and 75, 76 form the corresponding three turns. These results indicate that turns are well maintained in the peptide fragment as in the intact P2 protein. Each of the two turns formed by residues 56, 57 and 66-68, respectively, contains amino acid residues having high propensities for turn formation, Pro-56 in the first turn and Gly-67 in the second turn, as estimated with the method of Chou and Fasman (18).

There have been a number of studies designed to locate the antigenic determinant in the region of P2 protein (11,19-21). These peptides have displayed variations in the abilities of inducing EAN in Lewis rats by direct sensitization or by passive transfer of activated T cell lines. Synthetic peptides corresponding to the 57-81 (SP-25) and 61-72 (SP-12) amino acid sequence of the P2 protein have been shown to stimulate P2 protein-specific T cell lines and to transfer moderate and mild clinical signs of EAN, respectively (19,20). By direct sensitization, SP-25 can induce severe EAN while SP-12 was only able to induce mild signs of EAN. We have reported that SP-17 (residues 62-78) induces moderate clinical signs of EAN (11). In contrast, severe clinical signs of EAN has been induced in rats by sensitization with SP-26 or by SP-26 specific reactive T cells (21). These peptides are aligned in Fig. 3 together with the amino acid sequence of the antigenic region of P2 protein. Interestingly, the severity of clinical signs of EAN induced by different length of peptides well correlates with the length of the peptide and the number of turns. Only SP-26 encompassing the three turns show severe clinical signs of EAN, equivalent to those observed with the intact protein. Taken together these results suggest that there could be multiple epitopes in the antigenic region 53-78 of P2 protein and the turns could form the foci of these epitopes.

EAN and EAE are sister diseases of the nervous system. EAN produces inflammatory demyelination of the peripheral nerves while EAE causes demyelination of the nerve axons of the central nervous system. Both are T-cell mediated autoimmune diseases and both show strong similarities to paralysing diseases in humans, namely Guillain-Barre syndrome and multiple sclerosis respectively. The antigen for EAE is myelin basic protein (MBP) and various epitopes capable of producing autoimmune encephalomyelitis in animals of different species have been located in its sequence. Turns commonly exist in these EAE-inducing epitopes (7-10). Evidence for turns has also been found in MBP itself for both the guinea-pig (22) and rabbit (8) dterminants in using NMR spectroscopy. In addition, turn has been observed in an octapeptide which is part of an epitope to monoclonal antibodies to human MBP (23). It is therefore of considerable interest that turn structures also occur in an EAN-inducing peptide and these results suggest that turn structure could be a common structural motif for these closely related autoimmune neurological disorders.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Jeff Reimers for use of his curve-fitting and plotting programs. The research was supported by Sydney University Research Grant Funds.

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