Low-Ultraviolet Circular Dichroism Spectroscopy of Sequential Peptides 1–63, 64–95, 96–128, and 129–168 Derived from Myelin Basic Protein of Rabbit

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ABSTRACT: Four sequential peptides (sequences 1–63, 64–95, 96–128, and 129–168) derived from rabbit myelin basic protein by thrombic cleavage were examined by low-ultraviolet circular dichroism spectroscopy in 0.5 mM tris(hydroxymethyl)aminomethane hydrochloride (pH ~7.2) containing 0–92% trifluoroethanol (TFE). In the absence of the alcohol, all of the peptides contained a significant amount (17–29%) of β-structure. In the presence of relatively low concentrations (up to 30%) of TFE, all of the peptides except 96–128 adopted considerable α-helix (16–33%). This involved a transition from the β-structure in peptide 1–63 and transitions from the nonordered structure in peptides 1–63, 64–95, and 129–168. Furthermore, additional α-helix formed in peptide 1–63 between 30% and 92% TFE at the expense of nonordered structure, whereas the α-helix formation above 50% TFE in peptide 129–168 resulted largely from a β-structure → α-helix transition. With the exception of the 129–168 peptide, ~65–100% of the maximum level of β-structure persisted throughout the entire range of TFE concentration. In the case of peptide 129–168, however, most of the β-structure was converted to α-helix and nonordered structure at 75% TFE. While the present results support our previous assignments of β-structure- and α-helix-forming regions to specific amino acid sequences of the basic protein, they also demonstrate that the β-structure → α-helix transitions evidenced at various concentrations of TFE were influenced to a considerable degree by the length of the peptide, presumably due to the presence or absence of interactions between noncontiguous portions of the myelin basic protein polypeptide chain.

Myelin basic protein (MBP),[1] a major protein constituent of central nervous system myelin sheaths, has been extensively studied with regard to its immunological and physicochemical properties [for reviews, see Kies (1982) and Carnegie & Moore (1980), respectively]. Lipid-free MBP in dilute aqueous solution possesses hydrodynamic properties similar to those of flexible polyelectrolytes (Eylar & Thompson, 1969; Chao & Einstein, 1970; Kornguth & Perrin, 1971; Krigbaum & Hsu, 1975; Martenson, 1978; Mattice & Robinson, 1981) but becomes partially α-helical in the presence of anionic or zwitterionic amphiphiles (Anthony & Moscarello, 1971; Liebes et al., 1976; Smith, 1977; Keniry & Smith, 1979, 1981; Mendz & Moore, 1983; Mendz et al., 1984) or alcohols (Kornguth & Perrin, 1971; Block et al., 1973; Liebes et al., 1975; Swann & Li, 1979; Stone et al., 1985). MBP isolated from myelin in association with lipids has been reported (Riccio et al., 1984) to contain a significant amount of β-structure, and recent physicochemical studies have demonstrated the presence of 17% (Stone et al., 1985) to 25% (Randall & Zand, 1985) β-structure in the lipid-free protein.

As an approach to identifying amino acid sequences in the MBP that are capable of forming β-structure and α-helix under various solution conditions, we recently carried out studies (Stone et al., 1985) using low-ultraviolet circular dichroism (CD) spectroscopy on the two approximate halves (sequences 1–95 and 96–168) of rabbit MBP in 0–92% trifluoroethanol (TFE). The locations of the ordered conformations could be tentatively assigned within the amino acid sequence on the basis of hydropathic and predictive analyses. The present report describes similar studies carried out on two sequential peptides derived from each of the two larger portions of MBP. While largely confirming our previous assignments of sequences of β-structure and α-helix, the results also show that the secondary structure observed in the larger peptides resulted to some extent from interactions between noncontiguous portions of their chains as well as the independent contributions of the smaller peptides.

Experimental Procedures

Purified MBP component 1 from rabbit brain was prepared as described previously (Deibler & Martenson, 1973; Martenson et al., 1981). Peptides 1–63, 64–95, 96–128, and 129–168 were prepared by thrombic cleavage of the MBP (sequence 1–168) as described by Law et al. (1984). CD spectra were obtained on solutions of the peptides (~0.125 mg/mL, 20–40 μM) in 0.5 mM Tris-HCl buffer (pH 7.2–7.3) containing 0–92% TFE. The molar concentrations were accurately determined by amino acid analysis as previously described (Stone et al., 1985). The spectra were obtained and computer-analyzed to determine the percent contributions of α-helix, β-structure, β-turn, and nonordered conformations as described in detail by Stone et al. (1985). Five sets of the standard spectra derived from polypeptide and protein models were tested for correspondence between their best-fit composite spectra and the experimental CD curves. Results based on matrix 7 yielded the best-fitting analysis in most cases, as had been observed in the analyses referenced above. The percents of the various conformations according to matrix 7 are given with their standard errors in the legends to the appropriate figures. The two matrices discussed in this study were each

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[1] Abbreviations: CD, circular dichroism; MBP, myelin basic protein of rabbit; θ_r, molar residue ellipticity; TFE, trifluoroethanol; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

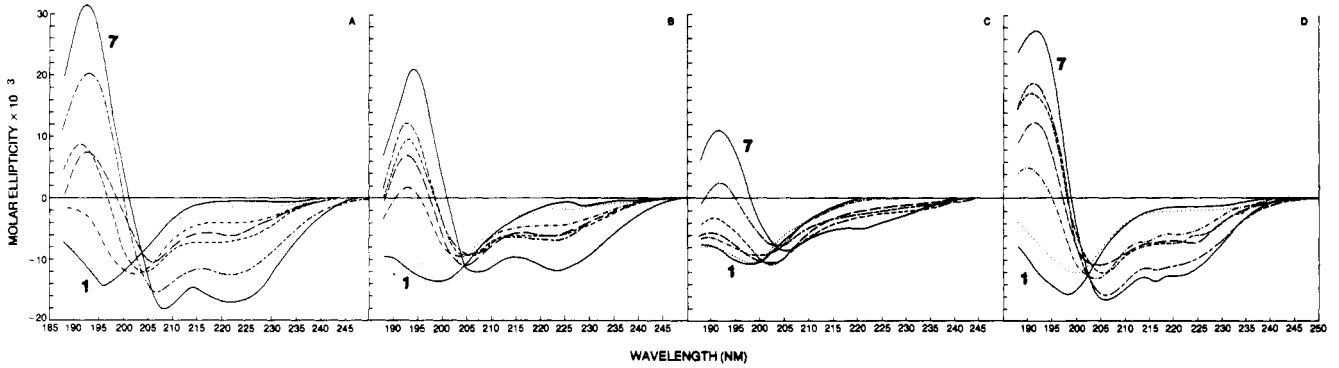

FIGURE 1: Low-ultraviolet CD spectra of sequential peptides derived from rabbit MBP in 0–92% TFE. (A) Peptide 1–63: (—, 1) 0% TFE; (···) 10% TFE; (-·-) 20% TFE; (--) 30% TFE; (---) 42% TFE; (----) 75% TFE; (–, 7) 92% TFE. (B) Peptide 64–95: same line code as in (A) at 0%, 10%, 20%, 30%, 50%, 75%, and 92% TFE. (C) Peptide 96–128: same line code as in (B). (D) Peptide 129–168: same line code as in (B).

composed of four standard spectra that were model spectra for various peptide conformations as follows: matrix 7, long α-helix, β-structure based on polypeptide models, class B spectrum of a β-turn (Woody, 1974), and extended nonordered structure based on polypeptide models; matrix 9, long α-helix, β-structure based on polypeptide models, class B spectrum of a β-turn, and a partially collapsed nonordered structure based on a polypeptide model.

The spectra of several of the solutions were taken in the Jasco 500J spectropolarimeter at a sensitivity of 1 mdeg/cm.

RESULTS

The four sequential peptides 1–63, 64–95, 96–128, and 129–168 display differences in the development of an α-helix-like CD spectrum as a function of increasing concentration of TFE (Figure 1A–D). A quantitative assessment of the nature of the conformations assumed by the four peptides as a function of the concentration of the alcohol was achieved by a best-fit analysis of the experimental spectra to standard CD spectra of four peptide conformations (α-helix, β-structure, β-turn, and nonordered structure) as described previously in the analysis of oligopeptides 1–95 and 96–168 (Stone et al., 1985). As in the previous study, one matrix (matrix 7) generally provided the best-fitting composite among the various sets of standards tested (Figures 2–5). In most cases, the agreement was good between the experimental points and these best-fit composite spectra. However, some discrepancy between the pairs of curves was observed, e.g., Figure 4, panels 6 and 7.

Matrix 7 is composed of spectra of the long α-helix, the β-structure of polypeptides, the class B β-turn of Woody (1974), and the extended nonordered polypeptide. The composite spectra based on other sets of standard spectra (Stone et al., 1985) indicated that neither the short α-helix ($n \sim 5$) nor a partially collapsed nonordered polypeptide provided an appropriate model for these peptides. The dotted line in Figure 2, panel 1, illustrates the particularly poor fit of the latter (matrix 9).

The percents of α-helix, β-structure, β-turn, and nonordered structure (matrix 7) in the four peptides as a function of the concentration of TFE are shown in Figure 6. Different patterns of conformational change in the increasing concentrations of TFE can be discerned among the four peptides by this analysis. Although lacking in α-helix in the absence of TFE, peptide 1–63 (Figure 6A) exhibits multiple transitions to α-helix in the presence of the alcohol. The strong tendency to form helical structures is evidenced by the transitions between 0% and 30% TFE, where the rise in helix between 0% and 20% TFE is accompanied by a diminution in β-structure, whereas the rise between 20% and 30% TFE derives from nonordered structure as well. The formation of α-helix between 42% and 92% TFE is primarily that of nonorder $\rightarrow$ order. About 23% of the 1–63 sequence is β-structure in the absence of TFE. Some of the residues comprising this structure apparently undergo a β-structure $\rightarrow$ α-helix transition as noted above, while an equivalent amount of β-structure is generated and lost during subsequent conformational changes in the peptide up to 92% TFE. About 30% of the sequence remains aperiodic at 92% TFE. There might be one stable β-turn within the peptide throughout the complete range of TFE concentration. However, this analysis assumed the major CD contribution from the β-turn would be that of a class B spectrum (Woody, 1974) so that a possible β-turn containing proline (e.g., Pro-Ser) would not have been counted; if present, its small CD component in the experimental spectrum (Hollosi et al., 1985) would have instead contributed to the deviations from the best-fit composite.

The conformational response of peptide 64–95 to increasing concentrations of TFE is shown in Figure 6B, where a nonordered structure $\rightarrow$ α-helix transition occurs between 10% and 30% TFE. There is a second transition to α-helix above 75% TFE. The extent of β-structure in this peptide is about 23% in the absence of TFE and increases to about 32% in 75% TFE. The magnitude of the changes in conformation from 75% to 92% TFE might be exaggerated due to an increase in the deviations of the best-fit analysis of the spectrum in 92% TFE. The small percent of the residues assigned to the β-turn conformation in this peptide would indicate a maximum of one β-turn within the sequence. However, if present, a possible proline-containing turn would have been counted with the α-helix.

The behavior of peptide 96–128 (Figure 6C) is striking in that there is virtually no tendency of the peptide to form a α-helix but rather a strong tendency to maintain a high content (28–37%) of β-structure throughout the entire range of TFE concentration, especially between 75% and 92%. A small but persistent amount of β-turn conformation is also apparent in this peptide.

In contrast with peptide 96–128, the C-terminal peptide 129–168 shows striking conformational changes (Figure 6D) that involve both the nonordered structure and the β-structure. The strong tendency of this peptide to form α-helices of up to 25% of its sequence is evident between 0% and 30% TFE, where the transition from the nonordered form predominates. Furthermore, there is a transition of approximately three-fourths of the β-structure ($\sim$19% of the total sequence) to α-helix and non-ordered structure. This occurs between 50% and 75% TFE and is followed by helix formation arising from

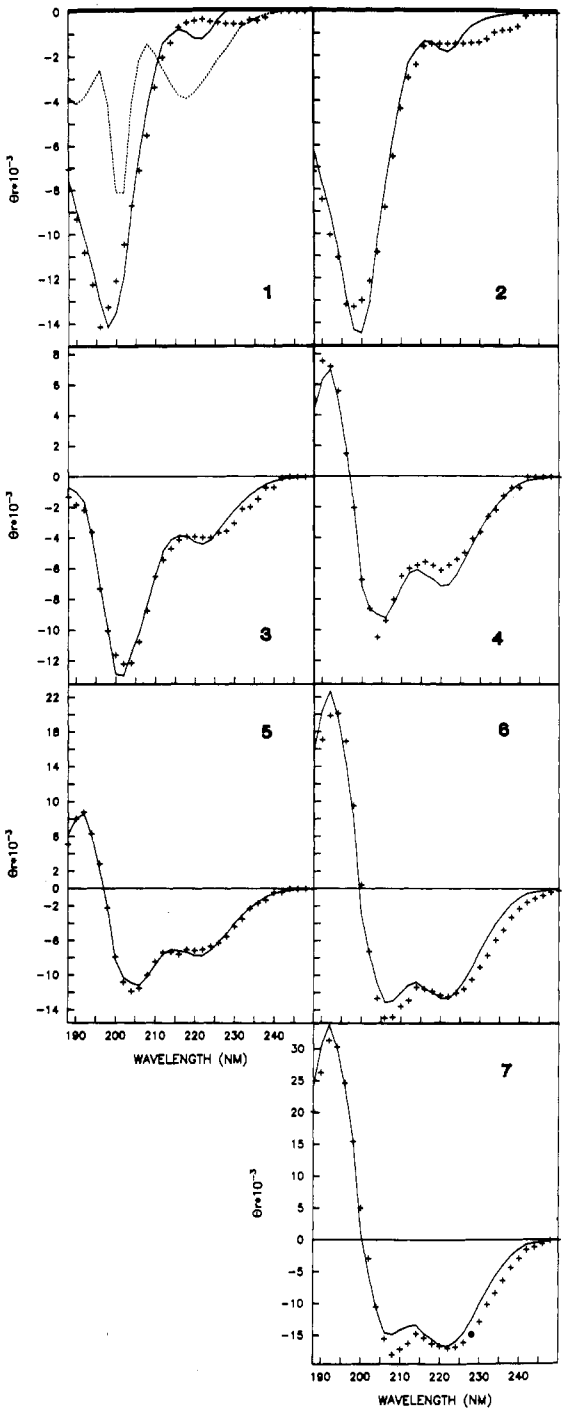

FIGURE 2: Best-fit analysis of peptide 1–63. Panels 1–7, 0–92% TFE, respectively. Matrix 7 computed curves, solid lines; experimental points, (+). The dotted line in panel 1 is the best-fit composite obtained with a partially collapsed polypeptide model (matrix 9). The percents of α-helix, β-structure, β-turn, and nonordered structure in the best-fit composites in panel 1–7, along with their standard errors, are respectively: (1) 0 ± 1, 23 ± 4, 4 ± 2, 73 ± 3; (2) 3 ± 1, 19 ± 4, 2 ± 2, 76 ± 3; (3) 12 ± 1, 17 ± 3, 0 ± 1, 71 ± 2; (4) 18 ● 1, 25 ± 4, 5 ± 2, 53 ± 2; (5) 21 ± 1, 21 ± 3, 1 ± 1, 57 ± 2; (6) 36 ± 3, 20 ± 11, 4 ± 4, 40 ± 7; (7) 50 ± 3, 16 ± 13, 7 ± 5, 27 ± 8.

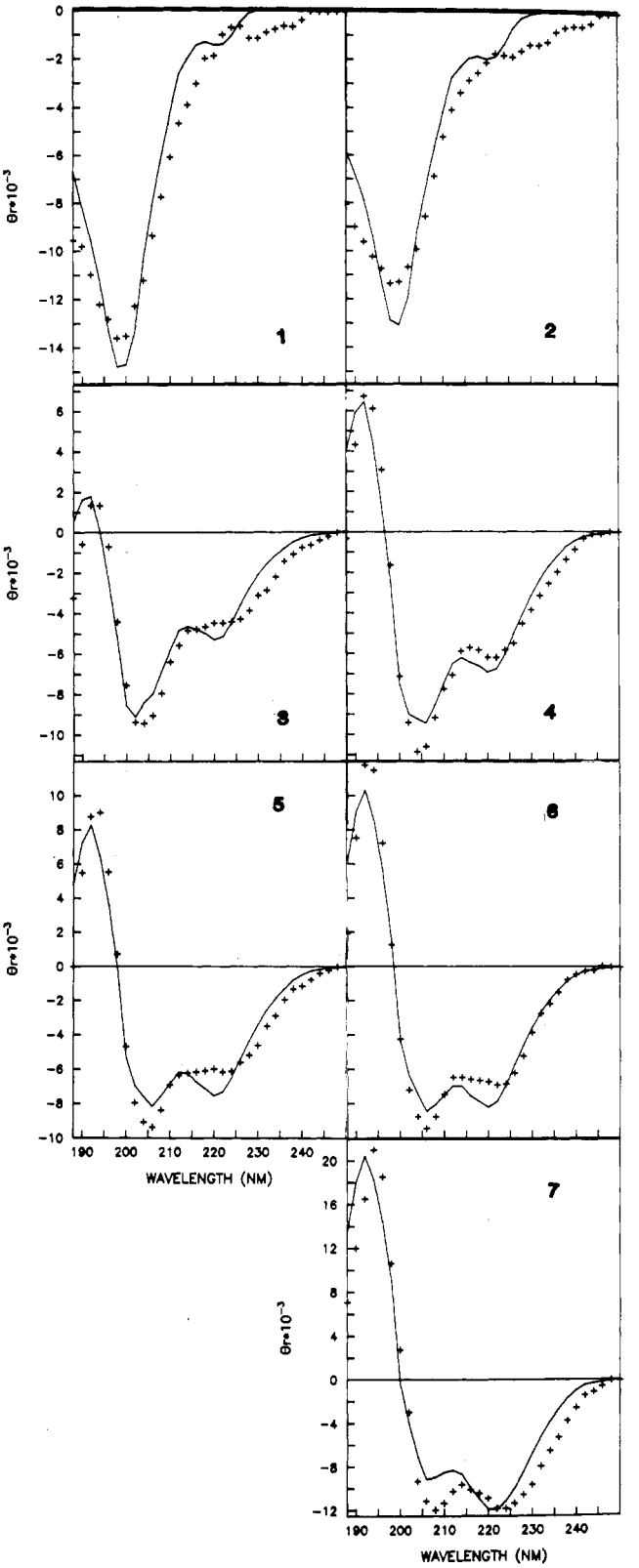

FIGURE 3: Best-fit analysis of peptide 64–95 represented as in legend to Figure 2: (1) 0.7 ± 2, 25 ± 8, 0 ± 3, 76 ± 5; (2) 1 ± 2, 26 ± 7, 1 ± 3, 71 ± 4; (3) 11 ± 2, 27 ± 7, 4 ± 3, 57 ± 4; (4) 16 ± 2, 26 ± 7, 3 ± 3, 54 ± 4; (5) 17 ± 2, 29 ± 8, 6 ± 3, 47 ± 5; (6) 18 ± 2, 32 ± 8, 6 ± 3, 44 ± 5; (7) 33 ± 4, 23 ± 16, 12 ± 6, 32 ± 10.

the nonordered structure between 75% and 92% TFE.

It is important to ascertain the relation of these conformational changes within the smaller peptides to those that occurred within the larger oligopeptides 1–95 and 96–168 (Stone et al., 1985). To this end, we have summed the number of amino acids assigned to the four conformations within the two pairs of smaller peptides and have compared each sum to the number present in the corresponding larger peptide (Figure 7). For simplicity, the residues in β-turns were taken to be in the nonordered conformation. Generally, the sums of each of the conformations in peptides 1–63 plus 64–95 (Figure 7A) yield distributions of amino acids in the α-helix, β-structure, and nonordered (including β-turn) conformations

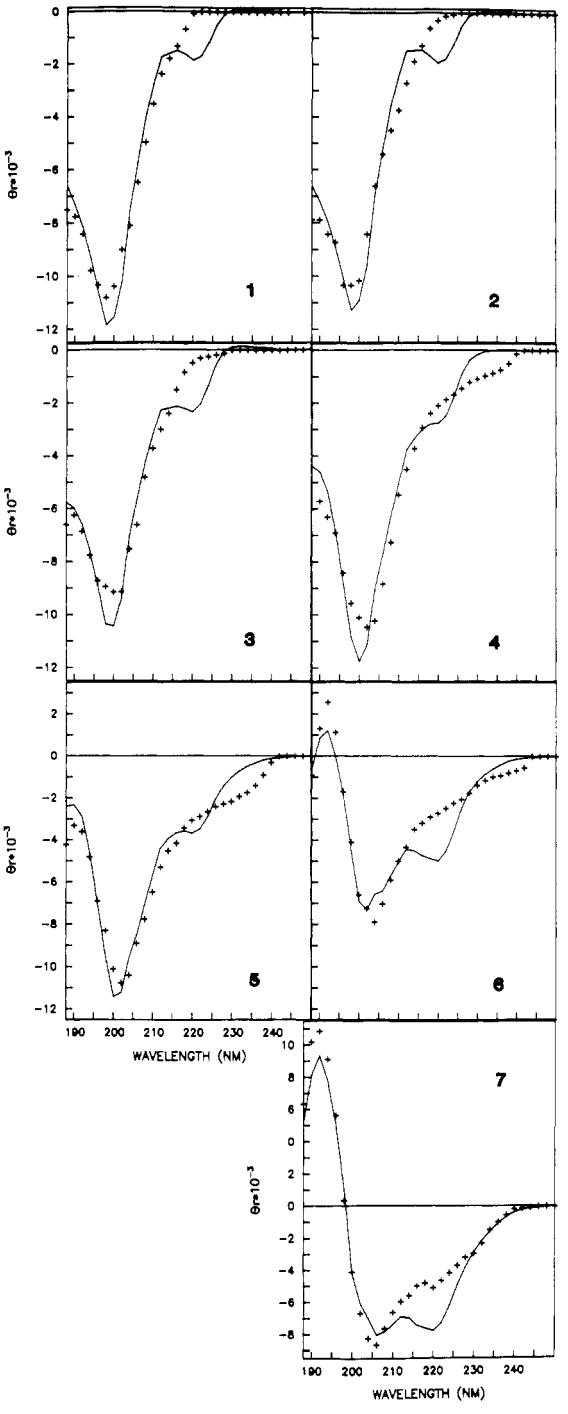

FIGURE 4: Best-fit analysis of peptide 96–128 represented as in legend to Figure 2: (1) 0 ± 1, 28 ± 5, 5 ± 2, 67 ± 3; (2) 0 ± 1, 29 ± 5, 6 ± 2, 65 ± 3; (3) 0 ± 1, 32 ± 5, 5 ± 2, 13 ± 3; (4) 2 ± 1, 31 ± 5, 1 ± 2, 67 ± 3; (5) 6 ± 1, 27 ± 5, 1 ± 2, 66 ± 3; (6) 7 ± 1, 36 ± 6, 5 ± 2, 52 ± 4; (7) 15 ± 2, 37 ± 8, 4 ± 3, 44 ± 5.

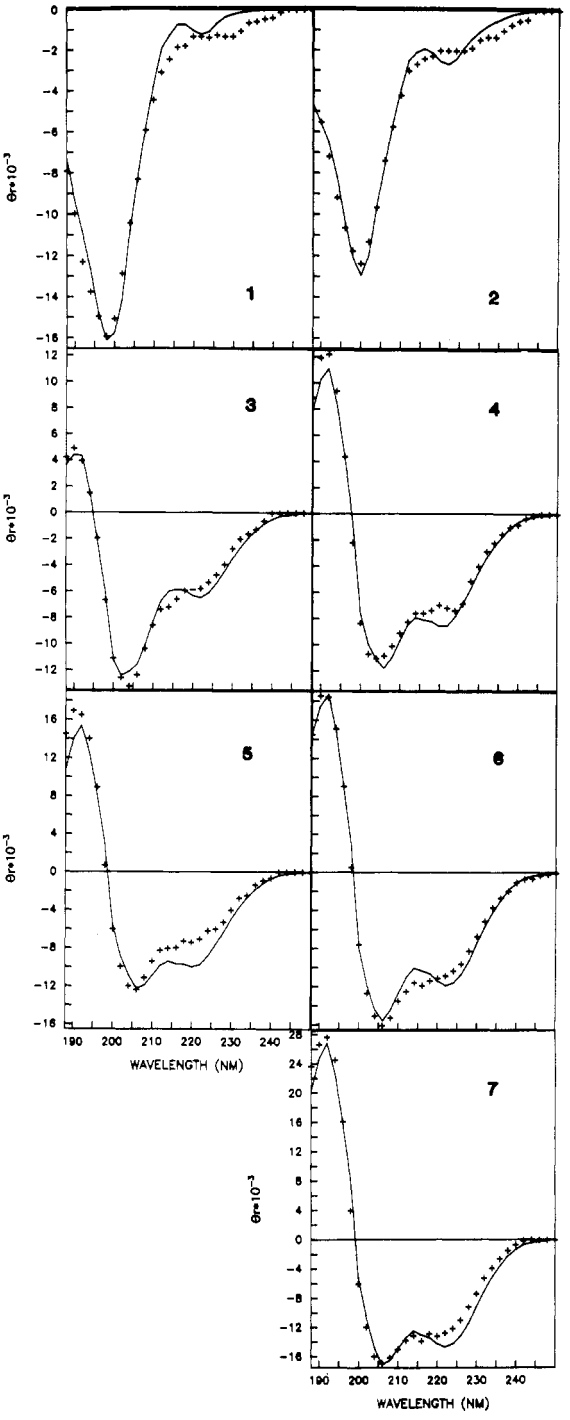

FIGURE 5: Best-fit analysis of peptide 129–168 represented as in legend to Figure 2: (1) 2 ± 1, 17 ± 5, 1 ± 2, 80 ± 3; (2) 5 ± 1, 19 ± 3, 4 ± 1, 71 ± 2; (3) 18 ± 1, 16 ± 4, 0 ± 1, 66 ± 2; (4) 23 ± 1, 22 ± 5, 1 ± 2, 54 ± 3; (5) 26 ± 2, 26 ± 10, 0 ± 4, 48 ± 6; (6) 37 ± 1, 7 ± 5, 0 ± 2, 56 ± 3; (7) 46 ± 2, 7 ± 10, 0 ± 4, 47 ± 6.

that are similar to those seen in peptide 1–95. However, the agreement is not perfect. In regard to the β-structure, its summation in peptides 1–63 and 64–95 between 0% and 30% TFE is somewhat greater than in peptide 1–95; the β-structures in the smaller peptides above 75% TFE are more stable than in the larger peptide, where they are largely converted to α-helix. In regard to the α-helix, more is formed between 20% and 92% TFE in peptide 1–95 than in the combination of the two derivative peptides. Although it is likely that the transition to α-helix previously observed in peptide 1–95 between 30% and 50% TFE was present in peptide 1–63 in the rise of α-helix between 42% and 75% TFE, the lack of an intermediate point in the rise precludes a definite statement about its midpoint. It is nevertheless clear that this transition did not occur in peptide 64–95.

Values for the summations of peptides 96–128 and 129–168 also show the same trends as the large peptide, i.e., 96–168 (Figure 7B). In this comparison, the major discrepancy is the greater content of β-structure in the large peptide relative to the summation of the two smaller peptides, except above 75% TFE where the discrepancy is reduced by the prominent β-structure $\rightarrow$ α-helix transition in peptide 96–168. The β-structure in sequence 96–128 is more stable in the shorter peptide than within peptide 96–168 and does not undergo this

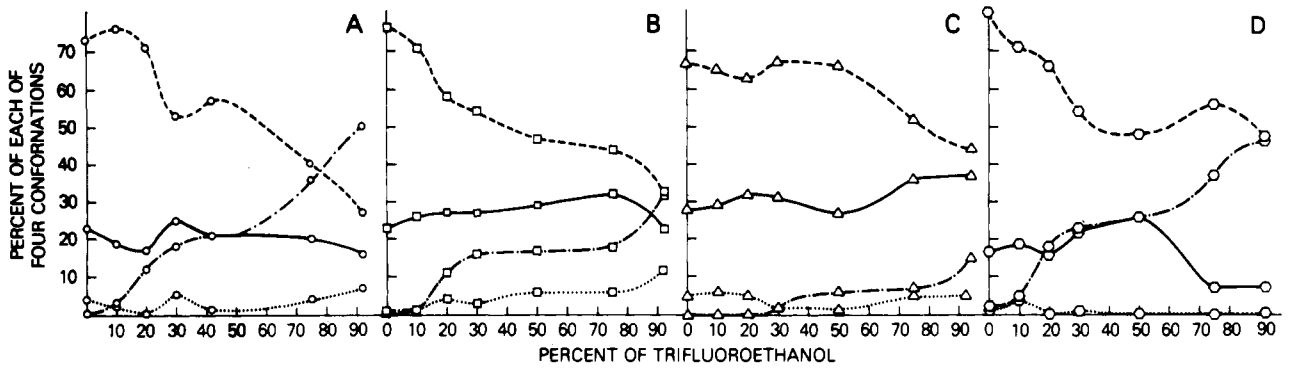

FIGURE 6: Percent of α-helix, β-structure, β-turn, and nonordered structure of the four sequential peptides in 0–92% TFE. (A) Peptide 1–63 (○); (B) peptide 64–95 (□); (C) peptide 96–128 (△); (D) peptide 129–168 (○). (–·–) α-Helix; (—) β-structure; (···) β-turn; and (– – –) nonordered structure.

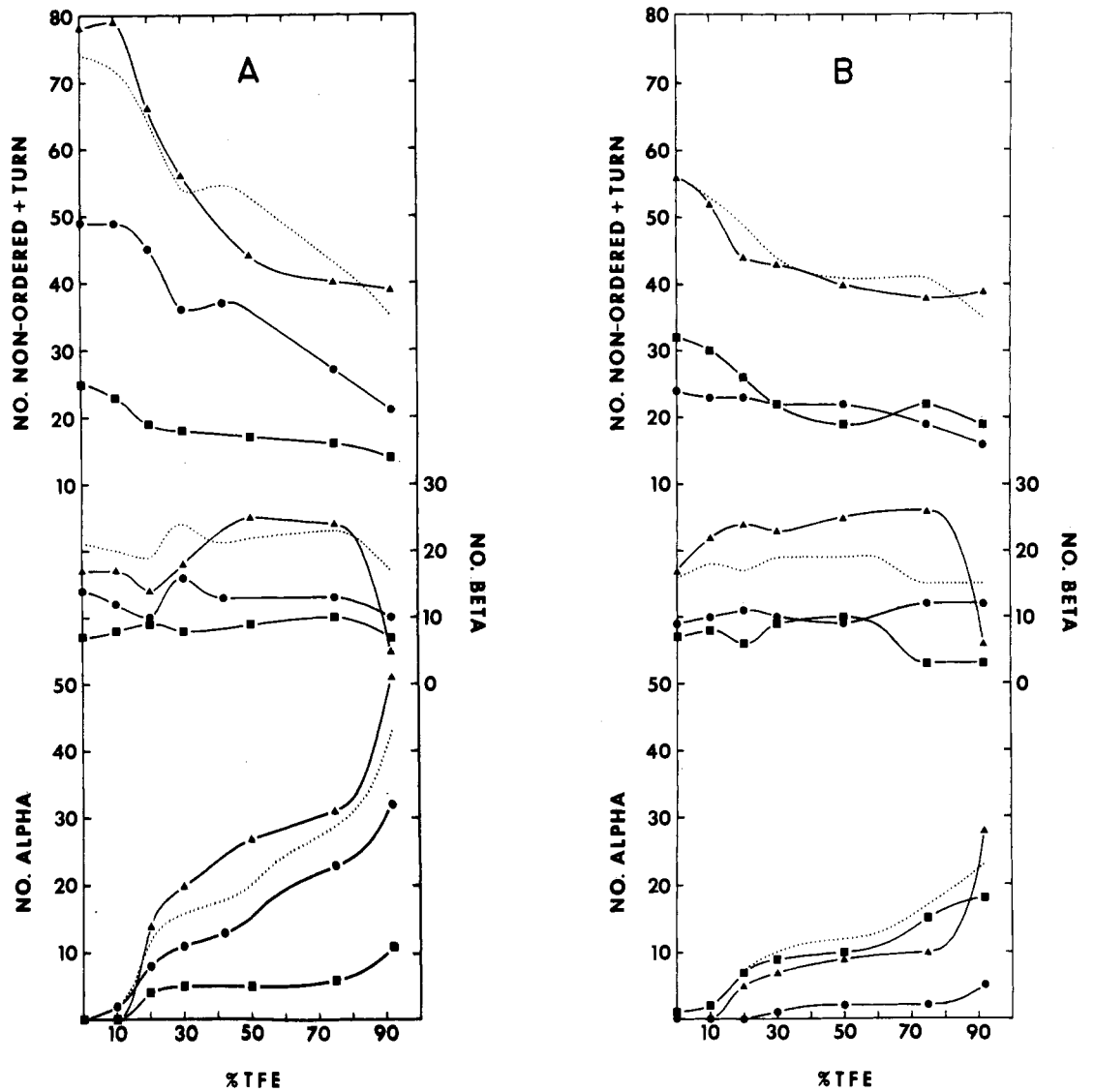

FIGURE 7: Number of amino acids in α-helix, β-structure, and nonordered structure plus β-turn. (A) N-Terminal half of rabbit MBP: (●) peptide 1–63; (■) peptide 64–95; (▲) peptide 1–95; (···) sum of peptides 1–63 and 64–95. (B) C-Terminal half of rabbit MBP: (●) peptide 96–128; (■) peptide 129–168; (▲) peptide 96–168; (···) sum of peptides 96–128 and 129–168.

transition up to 92% TFE, whereas the β-structure in sequence 129–168 is relatively less stable in the shorter peptide, converting to α-helix between 50% and 75% TFE.

Discussion

The conformation of MBP ultimately depends upon its amino acid sequence and the environment to which it is exposed. An analysis of the conformation of four sequential peptides derived from MBP by thrombic cleavage has been undertaken in order to identify amino acid sequences that contribute to the secondary and tertiary structure of the protein and to the conformational changes that may occur upon its incorporation into the myelin sheath. In a previous report (Stone et al., 1985), we examined the conformations of two

approximate halves of the protein as a function of increasing concentration of TFE by performing best-fit analysis on the low-ultraviolet CD spectra of the various systems. Both peptides 1–95 and 96–168 contained a significant amount of β-structure in the absence of the alcohol (as did the intact protein). In the presence of TFE, both underwent transitions to the α-helix from the nonordered conformation. Furthermore, two obvious β-structure → α-helix transitions were noteworthy. One took place at high TFE concentrations (75–92%) in both peptides, whereas the other occurred in peptide 1–95 at low concentrations of TFE (10–20%).

Our previous tentative assignment of the locations of α-helices in peptide 1–95 placed the major helix that formed between 10% and 30% TFE in the region of residues 7–29. The decrease in β-structure that occurred between 10% and 20% TFE was considered to have resulted from a β-structure → α-helix transition within sequence 15–21. Formation of additional α-helix between 30% and 75% TFE was ascribed to nonorder → order transitions occurring in sequences 58–63 and 70–75, whereas the helix formed between 75% and 92% TFE was ascribed to major β-structure → α-helix transitions within sequences 35–45 and 84–95. These assignments have been supported in part by the behavior of peptide 1–63. This peptide showed helix formation (~11 residues) between 0% and 30% TFE, accompanied by a decrease in β-structure between 0% and 20% of the solvent. Between 30% and 75% TFE, ~12 additional residues became helical; these can be accounted for by an extension of the first helix together with formation of an additional helix in sequence 58–63. The major β-structure → α-helix transition that occurred in peptide 1–95 between 75% and 92% TFE did not proceed beyond ~30% in either of the constituent peptides 1–63 or 64–95; instead, the increase in α-helix (approximately nine residues in peptide 1–63) was largely at the expense of nonordered structure. In peptide 64–95, the α-helix formed between 10% and 30% TFE (approximately five residues) can be tentatively assigned to sequence 70–75, Leu-Pro-Gln-Lys-Ser-His.

In the case of peptide 96–168, we assigned the α-helix that formed between 0% and 30% TFE (at the expense of nonordered structure) to residues 129–140 and the additional helix that formed between 75% and 92% TFE (at the expense of β-structure) to sequences 106–116 and 144–154. The present data obtained with peptide 129–168 show similar types of transitions and provide direct experimental support for the formation of helices within sequences 129–140 and 144–154. In comparison to peptide 96–168, however, peptide 129–168 formed the second helix over a lower range of TFE concentration (50–75% instead of between 75% and 92% TFE), and peptide 96–128 did not exhibit any such β-structure → α-helix transition (Figure 7).

In our previous study (Stone et al., 1985), we proposed that the β-structure initially present in peptide 1–95 consisted of a three-stranded antiparallel sheet formed by association of parts of the three hydrophobic sequences, 15–21, 37–45, and 84–92, and that in peptide 96–168 the β-structure consisted of a 2-stranded antiparallel sheet (or ribbon) formed by association of the hydrophobic sequences 106–112 and 148–154. According to these assignments, β-sheet formation would have involved interactions between hydrophobic regions well separated in the primary structure. In peptides 1–63, 64–95, 96–128, and 129–168, most of these interactions would be absent. The shorter peptides, however, could have formed short β-sheets by the folding back of single hydrophobic sequences near their middle [see, for example, Mattice & Scheraga (1985)]. [At the concentration of peptide used in the CD studies (~0.125 mg/mL), intermolecular β-sheet formation would be highly unlikely.] Depending upon the concentration of TFE, these short folded structures, which would be stabilized by interactions between regions close by in the linear amino acid sequence, could be either more or less resistant to transitions to α-helix than the longer two-stranded sheets formed by interactions between distant parts of the polypeptide chain within peptides 1–95 and 96–168. Specifically, the putative sheet formed in peptide 1–95 (Stone et al., 1985) by antiparallel alignment of the sequences Asp-Thr-Gly-Ile-Leu-Asp-Ser-Ile-Gly-Arg-Phe-Phe (34–45) and Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg (84–95) may be less stable in 92% TFE than a single, somewhat longer sequence, Arg-Asp-Thr-Gly-Ile-Leu-Asp-Ser-Ile-Gly-Arg-Phe-Phe-Ser-Ser-Asp (33–48), folded back in peptide 1–63 by a β-turn (possibly Asp-Ser-Ile-Gly). It may also be less stable than sequence 84–95 folded back in peptide 64–95 by a β-turn (possibly Phe-Lys-Asn-Ile). Similarly, sequence Arg-Gly-Thr-Val-Leu-Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu (105–117) in peptide 96–128 may retain a more stable β-sheet in 92% TFE if folded back on itself than if associated antiparallel with sequence Ala-Gln-Gly-Thr-Leu-Ser-Arg-Leu-Phe-Lys-Leu (144–154) in peptide 96–168. Conversely, a sheet formed by the folding back of sequence 144–154 in peptide 129–168 may be less stable, undergoing a transition to α-helix above 50%, rather than above 75%, TFE. From the point of view of helix stability, a system containing two or more long helices that could interact, as in peptide 1–95, 96–168, or 129–168, should be more stable than a system containing a single long helix or very short interacting helices, as would be the case in peptides 64–95 and 96–128. On this basis, the absence of significant β-structure → α-helix transitions in the latter two peptides might be clarified. However, numerous factors would contribute to the observed relative stabilities of the different conformations within the short vs. the long oligopeptides in varying concentrations of aqueous TFE solutions. In addition to electrostatic interactions and solvation reactions involving both water and TFE, the specific types of interactions believed to occur between water and either given amino acid residues or larger groups of residues [e.g., see Berendsen (1975) and Eagland (1975)] would be expected to play an important role.

A comparison of the numbers of residues in the four conformations in the intact MBP in 0% and 92% TFE with the corresponding numbers obtained by summing the conformations of peptides 1–95 and 96–168 showed excellent agreement (Stone et al., 1985). Analyses in progress of the CD spectra of MBP at the intermediate concentrations of TFE may permit a comparison of the conformational transitions of the intact protein with those of its various sequential peptides. In some cases of the MBP spectra, however, the best fits of the composite curves to the experimental points have been less satisfactory than those of the peptides and require further investigation. Preliminary results indicate that the intact protein has a greater tendency to form α-helices than does its peptides and that it more readily undergoes β-structure → α-helix transitions.

This study and the previous one (Stone et al., 1985) have shown that several regions of MBP, whether part of the intact protein or present in oligopeptide fragments, have a strong tendency to adopt β-structure in aqueous solution. These regions have been tentatively assigned to the five most hydrophobic segments of the polypeptide chain, which have a high statistical probability of lying in a β-sheet. Incorporation of these regions in an appropriate manner into a β-sheet would create a hydrophobic core, which, though small, would be

capable of generating a specific polypeptide chain topology and tertiary structure in the protein. In a moderately hydrophobic environment, several regions, which can be tentatively identified on the basis of secondary structure prediction algorithms, adopt the α-helical conformation. These helices arise as a result of transitions from both nonordered conformations and β-structure. Thus, the possibility exists that in vivo the newly synthesized polypeptide chain could fold into a specific tertiary structure in which the β-sheet and nonordered form predominate. Subsequent incorporation of the MBP into the $\sim$30-Å aqueous space separating the cytoplasmic surfaces of the central nervous system myelin lamellae (Kirschner et al., 1984) could be accompanied by conformational transitions involving α-helix formation in certain regions of the molecule. The resulting tertiary structure, consisting in part of a small β-sheet and several α-helices, could stabilize the myelin lamellae by electrostatic interactions with the head groups of acidic lipids (Mattice & Robinson, 1981) as well as provide hydrophobic contacts for possible MBP dimerization across the cytoplasmic space (Braun, 1984).

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