Low-Ultraviolet Circular Dichroism Spectroscopy of Oligopeptides 1-95 and 96-168 Derived from Myelin Basic Protein of Rabbit

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ABSTRACT: Myelin basic protein (MBP) is a major protein constituent of the myelin sheath of the central nervous system, where it is believed to have functional α-helical segments. One element of the function of the protein might be "conformational adaptability" of specific regions of its amino acid sequence, since the purified protein appears to be largely devoid of ordered structure. To pursue this question, low-ultraviolet circular dichroism (CD) spectroscopy was conducted on the sequential thrombic peptides 1-95 and 96-168 of the protein in the presence of 0-92% trifluoroethanol (TFE), a solvent known to promote stable secondary structures in polypeptides. The series of CD spectra of the oligopeptides were subjected to a computerized best-fit analysis of four peptide conformations, the α-helix, β-structure, β-turn, and nonordered form. Agreement between experimental and best-fit composite spectra was achieved when standard CD curves of peptide conformations were derived from known theoretical spectra and experimental spectra of polypeptides. In dilute buffer alone, oligopeptides 1-95 and 96-168 evidenced no α-helix but significant β-structure (18% and 23%, respectively), as well as a predominant, extended nonordered conformation. However, the two parts of the protein differed in conformational adaptability. From 0% to 30% TFE, 96-168 exhibited concomitant transitions to 10% helix and 32% β-structure from the nonordered form. In contrast, in 10-30% TFE, 1-95 underwent a transition to ~21% helix with partial loss of β-structure as well as nonordered form; higher concentrations of TFE (40-75%) promoted additional transitions to both helix and β-structure (totaling 33% and 25%, respectively). A maximum of ~50-55% of the amino acid residues of both oligopeptides ultimately resided in sequences of ordered structures from 50-92% TFE. In contrast, ~45% of their amino acids retained the nonordered conformation apparently by virtue of sequences that contain up to 50% Pro, Gly, Ser, and Asp. The locations of the ordered conformations could be tentatively assigned within the amino acid sequence on the basis of hydropathic and predictive analyses. β-Structure $\rightarrow$ α-helix transitions occurred between 75% and 92% TFE, which apparently rendered the ordered sequences mainly helical. These patterns of conformational change underscore the involvement of β-structure and highly adaptable amino acid sequences in the conformations of MBP.

Myelin basic protein, a major protein component of central nervous system myelin and the causative agent of autoimmune encephalomyelitis (Kies et al., 1958; Laatsch et al., 1962), is located in the interbilayer space between the cytoplasmic surfaces of the lamellae formed by the oligodendroglial plasma membrane (Poduslo & Braun, 1975; Golds & Braun, 1976; Omlin et al., 1982). Here, strong interaction between the protein and the polar head groups of the lipids is believed to stabilize the compact lamellar structure of the myelin sheath (Sedzik et al., 1984). Investigations of the structure of myelin basic protein by various physicochemical techniques [see review by Carnegie & Moore (1980) and also by Anthony & Moscarello (1971), Kornguth & Perrin (1971), Swann & Li (1979), Keniry & Smith (1981), Mendz & Moore (1983), and Mendz et al. (1983, 1984)] have shown that the protein is highly solvated and largely nonordered in dilute aqueous solution but tends to form α-helical structures under certain environmental conditions. By utilizing interactions between various fragments of myelin basic protein and lipid vesicles or micelles, Keniry & Smith (1981) and Mendz et al. (1984) have provided evidence of a major helix-forming region encompassing the Phe-Phe sequence near the center of the polypeptide chain.

The objective of the present study was to determine the adaptation of thrombic oligopeptides of myelin basic protein of rabbit (MBP)[1] to the physicochemical forces exerted upon them in systematically modified aqueous environments. In particular, we wished to observe whether and how they adopt favored stable conformations in the presence of an alcohol that would promote such intramolecular stabilization, e.g., trifluoroethanol (TFE) (Eagland, 1975). The ultimate goal is to understand the secondary structure of the complete protein and the "conformational adaptability" of myelin basic protein to its environment in biological systems. To this end, we have employed low-ultraviolet circular dichroism (CD) spectroscopy of sequential oligopeptides of MBP in aqueous solutions of increasing percent of TFE. Utilizing various sets of CD spectra of the α-helix, β-structure, β-turn, and nonordered conformations of peptides and proteins within a computer program of best-fit analysis, we were able to select appropriate standard spectra and determine the nature and degree of the conformations adopted by the peptide bonds of the oligopeptides derived from the protein. This report shows the different conformational properties of the N-terminal and C-terminal

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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.

halves of the protein (residues 1–95 and 96–168). Similar analyses of the smaller enzymatic cleavage products composing each half will be presented subsequently.

Experimental Procedures

MBP and the 1–95 and 96–168 Peptides. Preparation of purified MBP component 1 from rabbit brain was carried out as described previously (Deibler & Martenson, 1973; Martenson et al., 1981). Component 1 is the MBP species that contains an intact C terminus; it is not phosphorylated or deamidated (Deibler et al., 1975). Thrombic cleavage of the MBP at the Arg^{95}–Thr^{96} bond and the separation and purification of the cleavage products have been described (Law et al., 1984). Since the amino acid sequence of the protein was known (Martenson et al., 1981; Deibler et al., 1985), the molar concentration of the MBP and oligopeptides could be accurately determined by amino acid analysis as an average of the molar concentration derived from each of the acid-stable amino acids.

Measurement of the Circular Dichroism Spectra. All chemicals were of the highest purity grade. The TFE was spectral grade from MANN. Aliquots of the oligopeptide of about 1.9 mg, as determined by the amino acid analysis, were placed in vials and lyophilized for storage until use. Stock solutions were then prepared by adding to the vial 680 μL of glass-distilled water containing dilute Tris·HCl buffer (0.5 mM, pH 7.2–7.3) to maintain the pH above 7. Subsequently, 90 μL of the stock solution was added to appropriate amounts of buffer followed by TFE to achieve a total volume of 1.95 mL of 0%, 10%, 20%, 30%, 50%, 75%, and 92% TFE. The CD spectrum of the sample (about 0.125 mg/mL unless otherwise stated) was measured at 25 °C in a high-quality quartz optical cell of 0.5-mm path length. The spectrum was taken from 300 to 186 nm in a Cary 60 spectropolarimeter that had been updated by placing the Pockel cell in a fixed position in the light path in front of the sample optical cell and by installing a larger phototube (modification by Jack Aviv). The full-scale sensitivity was usually 0.04° with the longest pen period of 10 between 230 and 185 nm. The noise levels in θ near the wavelengths of the various CD peaks averaged around ±150, 330, 800, and 1080 at 220, 205–207, 197, and 192 nm, respectively. No refractive index correction was made.

Analysis of CD Spectra. To determine the conformation of the oligopeptides, the best fits of various matrices (each composed of four model CD spectra representing the α-helix, β-structure, β-turn, and nonordered conformations) to the experimental CD curve were achieved by the use of a computer program in the M lab System at the National institutes of Health (Knott, 1979). The matrices were tested for their ability to provide a quantitative agreement between the best-fit composite spectrum and the experimental curve. These analyses employed molar residue ellipticities at 2-nm intervals between 250 and 186 nm. The program is available upon request.

In regard to the selection of the standard CD spectra, we tested those model CD spectra that were derived previously by three essentially different rationales. Experimental CD spectra of various synthetic homopolypeptides have been obtained under conditions where they adopted the above conformations [e.g., see Holzworth & Doty (1965), Carver et al. (1966), Davidson & Fasman (1967), Greenfield et al. (1967), Timasheff & Gorbunoff (1967), Quadrifoglio & Urry (1968), Urry et al. (1974), Brahms et al. (1977), Brahms & Brahms (1980), and Gierasch et al. (1981)]. The theoretical CD spectra of the α-helix (Moffit, 1956) and β-turn (Woody, 1974) peptide conformations have also been calculated. Finally, the CD spectra of proteins having known conformations as determined by X-ray crystallography have been resolved into the component CD contributions of their α-helical, β-structure, β-turn, and nonordered sequences [model spectra, on the basis of averages of up to 14 proteins, that are considered to be more representative of the CD of the peptide bonds in globular proteins (Saxena & Wetlaufer, 1971; Chen et al., 1972, 1974; Chang et al., 1978)]. Although the three approaches have yielded the same, widely used spectrum of the "infinite" α-helix, there were significant differences in the model CD spectra derived for the other conformations. Thus, quantitative interpretation of protein conformation by this type of analysis must be considered limited unless tested or refined. A preliminary best-fit analysis of an independent protein system enabled the evaluation of the various standard CD spectra; those yielding poor correspondence with experiment were readily eliminated from further consideration (e.g., the "remainder" spectrum derived from actual proteins). The experimental CD spectra of polypeptides that had been induced to form α-helix, β-structure, and "collapsed" nonordered conformations in the presence of acidic polysaccharides in solution (Stone & Epstein, 1977; Stone & Smith, 1982) were included with the remaining model spectra cited above (Table II, Appendix) as possible standards of the four conformations. The spectra were compiled in various matrices that allowed the comparisons necessary to achieve confidence in the uniqueness of the best-fit spectrum and in its actual correspondence to the experimental spectrum. Numerous analyses of the CD spectra of the oligopeptides and MBP in the various solvents showed that the spectral standards based on the polypeptide models afforded a greater degree of correspondence to the experimental spectrum than did the analogous spectra derived from actual proteins. Furthermore, the best-fitting composite curves were achieved by means of the same one of 10 varying matrices in all cases (matrix 7, Appendix). As will be shown under Results, this set of standards provided good agreement with experiment; the data obtained by this means were utilized in the determination of the conformations of the oligopeptides and MBP.

As an estimation of the CD contribution of the possible β-turns in the oligopeptides, the theoretical class B β-turn spectrum computed by Woody (1974) was utilized in all 10 matrices. This assumption was made because the class B spectrum is considered to be the most common CD spectrum associated with the various types of β-turn conformations (Woody, 1974; Smith & Pease, 1980). However, further studies should examine the quantitative effect of this assumption on the best-fit composites (for further discussion, see the Appendix).

Results

The oligopeptides of MBP displayed increasing alteration in their CD spectra in increasing percent of the TFE solvent (Figure 1). These spectra change in a manner similar to that reported previously (Liebes et al., 1975) for the intact bovine MBP and indicate an increase in α-helical structures. Differences between the response of the N-terminal and C-terminal halves of the protein to TFE emerge upon comparison of panels A and B.

Best-Fit Analysis. For each spectrum in Figure 1A–C, the best-fit analysis showed good agreement with experiment (Figures 2 and 3). These were obtained with the same matrix in all cases (matrix 7, Appendix). This was composed of the CD spectrum of the β-structure and the extended nonordered

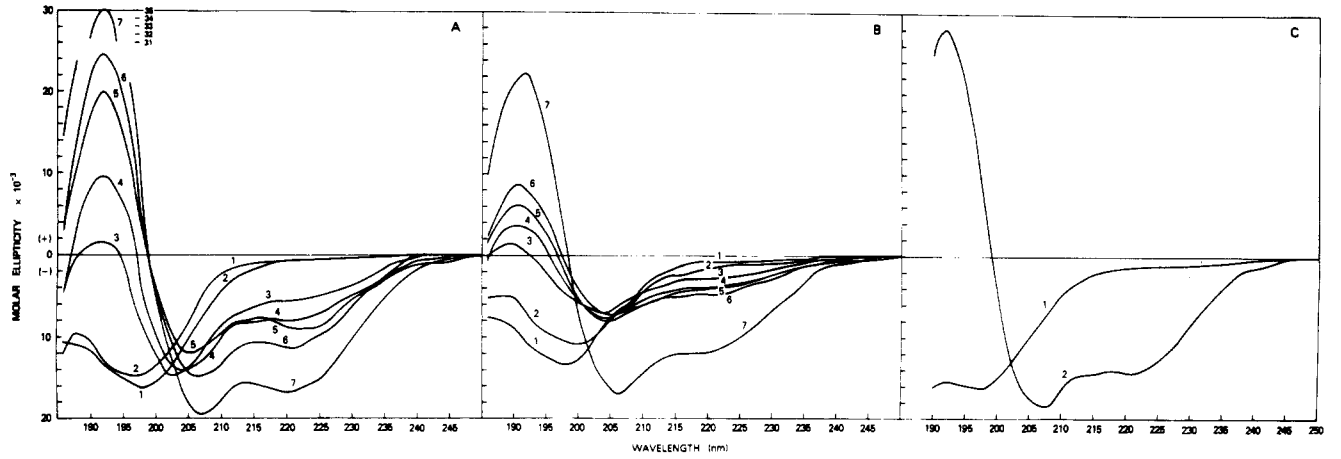

FIGURE 1: Low-ultraviolet CD spectra of MBP and MBP-derived oligopeptides in 0–92% TFE. (A) Peptide 1–95: curves 1, 2, 3, 4, 5, 6, and 7 represent 0%, 10%, 20%, 30%, 50%, 75%, and 92% TFE, respectively. (B) Peptide 96–168: same line code as for (A). (C) MBP: curve 1, 0% TFE; curve 2, 92% TFE.

form of polypeptide models, the long α-helix, and the class B spectrum of β-turn.

The presence of the extended rather than the partially collapsed nonordered structure in these oligopeptides was evidenced by virtue of the drastic reduction in agreement when the spectrum of the partially collapsed form was used (e.g., Figure 3, panel 1). This distinction was less sharp at high concentrations of TFE (75% and 92%) (Figure 2, panel 7) where the percent of nonordered form was greatly diminished (42–45% from 72–83%). A degree of partially collapsed segments would tend to decrease the computed percent of β-structure while increasing the percent of the β-turn. These oligopeptides also appeared to form helices of $n \geq 7$ since the spectrum of the long α-helix afforded better "best fits" than that of the short helix, which was distinctly poor in many of the cases (e.g., Figure 3, panel 7).

Conformations of the Oligopeptides. Agreement between the experimental and best-fit composite curves of peptide 96–168 (Figure 3) was good, with standard deviation(s) (SD) of 1–2% for the α-helix, 4–7% for the β-structure, 2–3% for the β-turn, and 3–5% for the nonordered extended conformation (see Table III, Appendix). The higher SD were all associated with the spectrum at 92% TFE. Agreement between experiment and the best-fitting curves of peptide 1–95 (Figure 2) was also good, but the SD values were higher than for peptide 96–168 (2–3%, 7–12%, 3–5%, and 5–8% for the respective conformations). Best-fitting curves in the case of MBP exhibited similar SD values.

The change in conformations of the two oligopeptides as a function of the percent TFE pointed out a difference in their tendency to form α-helical structure in this solvent (Figure 4A). Here, the curve for the 96–168 peptide displays a simple increase from 0% to 10% helix as the TFE goes from 0% to 30%. The helicity increases slightly up to 75% TFE, after which a sharp rise in helix content reaches 39% in 92% TFE. In contrast, the curve for the 1–95 peptide appears to undergo at least two conformational transitions between 0% and 75% TFE. The first transition from 0% to 21% α-helix occurs between 10% and 30% TFE. The second transition occurs between ~30% and ~70% TFE, where the degree of helicity reaches 33%. Again, there is an additional, sharp transition which in this case leads to 54% helix at 92% TFE. On the basis of these data alone, it would appear that the two portions of the protein had in common a tendency to form relatively stable α-helix in part(s) of the chain but that the 1–95 peptide could form relatively twice as much. In addition, by ~70% TFE, peptide 1–95 could also be induced to form an additional ~12% helix that was relatively less stable.

Further insight into the nature of the difference between the two oligopeptides was gained by considering the behavior of the portions of their chains that did not undergo transitions to the α-helix conformation (Figure 4B,C). The curve of the β-structure of the 96–168 peptide (Figure 4B) exhibits a relatively smooth transition from 23% β-structure in the absence of TFE to 33% in 30% of the solvent whereupon the level of β-structure remains essentially unchanged up to 75% TFE. The pattern of this transition is, therefore, similar to that of the α-helix as both are concomitantly formed by loss of the nonordered form (Figure 4C). The precipitous decrease in the β-structure in 92% TFE, however, is concomitant with a sharp rise in α-helix content rather than an increase in nonordered form. The data for β-turns are not included in Figure 4; these are given in Table III (see the Appendix). Values of the percent β-turn are relatively low for the 96–168 peptide but more or less persistent throughout the 0–75% TFE range.

The behavior of the 1–95 peptide differed from that of the 96–168 peptide (Figure 4). The proportion of β-structure is lower (18% in 0 and 10% TFE), and it decreases between 10% and 20% TFE, coincident with the first rise in α-helix. Thereafter, there is a rise in percent β-structure to 25–26% at 50% and 75% TFE. As in the case of the 96–168 peptide, the sharp decrease in percent β-structure in peptide 1–95 in 92% TFE is accompanied by a rise in α-helix rather than by a change in nonordered conformations. Very few of the peptide bonds exhibited CD characteristics of the β-turn conformation.

The intact MBP exhibited essentially no α-helix and no β-turns but about 17% β-structure in the absence of TFE; in the presence of 92% TFE, the protein contained 47% helix, 6% β-structure, and no β-turns according to the best-fit analysis (Table III; see the Appendix). The calculated numbers of the amino acids in the four peptide conformations are given in Table I. Particularly noteworthy is the comparison between the intact protein and the sum of its two cleavage products. There is but slightly greater secondary structure in the sum than in MBP in the absence of TFE, and in 92% TFE the secondary structure present in the intact protein is equal to that obtained by summing its parts.

Discussion

Recently, MBP and its oligopeptides have been the focus of numerous investigations already cited regarding the con-

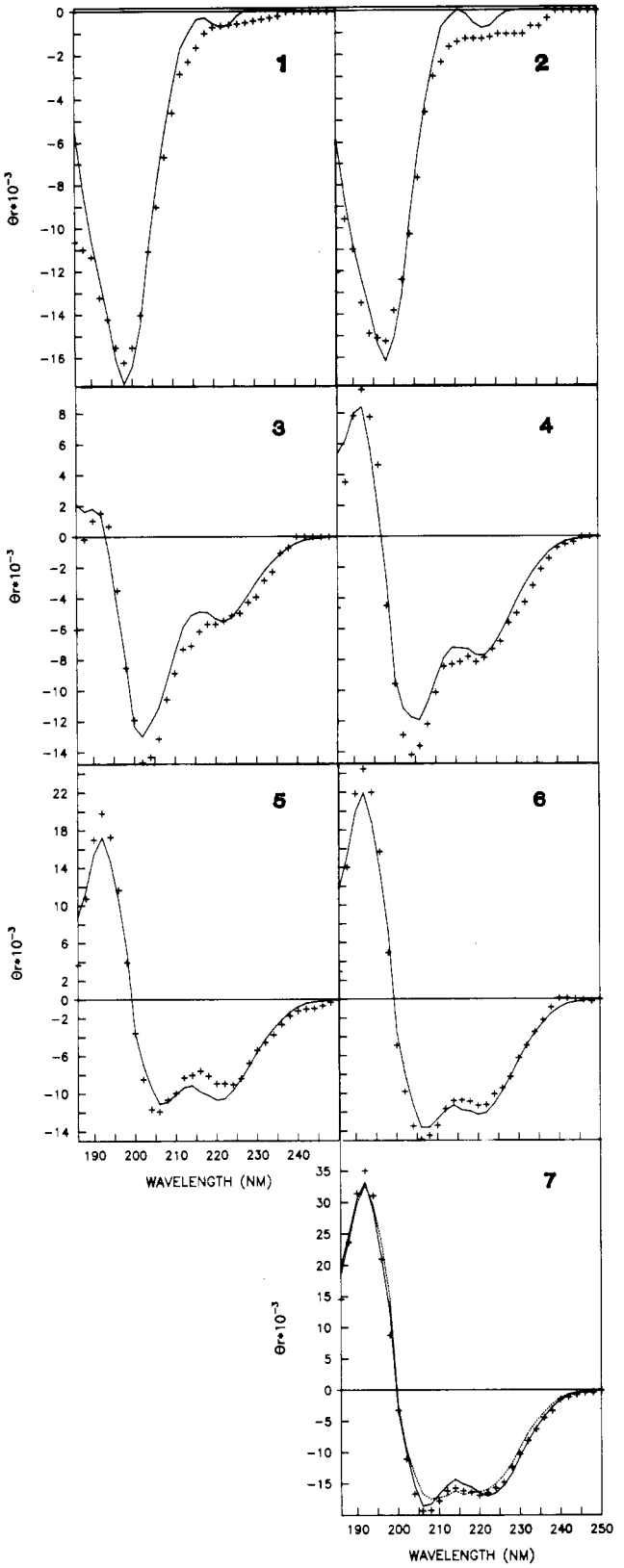

FIGURE 2: Comparison of the spectra generated by best-fit analysis with the experimental spectra of the 1–95 peptide in 0–92% TFE. (+) Experimental spectral points; (—) predicted curve of best fit to standard matrix 7; (···) (panel 7) best fit to standard matrix 9. Panels 1, 2, 3, 4, 5, 6, and 7, 0%, 10%, 20%, 30%, 50%, 75%, and 92% TFE, respectively.

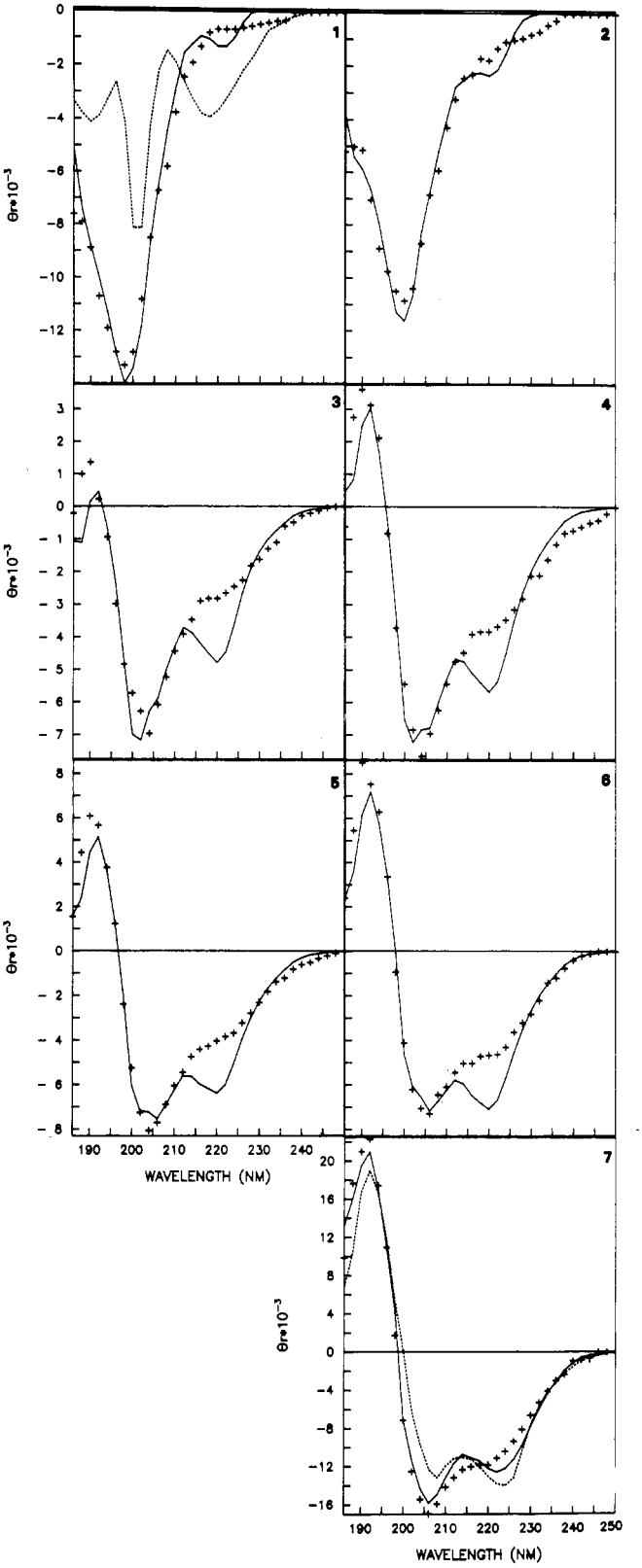

FIGURE 3: Analyses of 96–168 peptide. Comparison of the spectra generated by best-fit analysis with the experimental spectra in 0–92% TFE. (+) Experimental spectral points; (—) predicted curve of best fit to standard matrix 7; (···) (panel 1) best fit to matrix 9; (···) (panel 7) best fit to matrix 8. Panels 1, 2, 3, 4, 5, 6, and 7, 0%, 10%, 20%, 30%, 50%, 75%, and 92% TFE, respectively.

formational nature of select regions of the molecule and how these might play a role in the association of the protein with the plasma membrane, in the molecular interactions between MBP monomers, and in the intramolecular structure of MBP.

Furthermore, Mattice & Robinson (1981) have generalized [from their findings and those of Yang et al. (1977)] that certain brain proteins and oligopeptides such as MBP, lipotropin, and endorphin, which in aqueous solution in the purified state exhibit essentially no stable α-helix, might contain helical

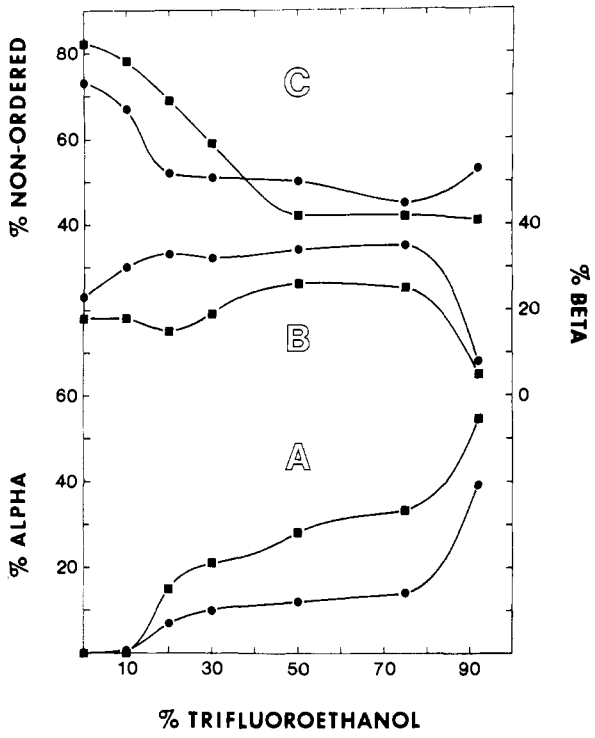

FIGURE 4: Percent helix, β-structure, and extended nonordered structure in MBP-derived oligopeptides in 0–92% TFE. (C) Extended nonordered structure; (B) β-structure; (A) α-helix; (■) 1–95; (●) 96–168.

Table I: Number of Amino Acids in the Four Conformations Calculated from Best-Fit Analysis Based upon Matrix 7 Standard Curves[a]

	no. of amino acids at TFE concn (%)						
	0	10	20	30	50	75	92
1–95 peptide							
α-H_L	0	0	14	20	27	31	51
β-P_{pp}	17	17	14	18	25	24	5
β-t	0	5	0	0	4	0	0
ENOS	78	74	66	56	40	40	39
96–168 peptide							
α-H_L	0	0	5	7	9	10	28
β-P_{pp}	17	22	24	23	25	26	6
β-t	3	2	5	5	4	5	0
ENOS	53	50	39	38	36	33	39
MBP							
α-H_L	1						79
β-P_{pp}	29						10
β-t	0						0
ENOS	138						79
1–95 + 96–168							
α-H_L	0						79
β-P_{pp}	34						11
β-t	3						0
ENOS	131						78

[a] Abbreviations: α-H_L, α-helix ($n > 7$); β-P_{pp}, β-structure; β-t, β-turn; ENOS, extended nonordered structure.

portions in the presence of the lipid bilayer of the plasma membrane. In the present study, we have utilized CD spectroscopy to analyze the conformational adaptability of MBP and its two halves derived by thrombic cleavage. We conclude that good correspondence with the experimental spectra could be achieved by means of a best-fit analysis employing certain model CD spectra of polypeptides (see the Appendix). Given this analysis, MBP and its two oligopeptides were shown to contain a significant proportion of stable β-structure in dilute aqueous buffer while exhibiting no α-helix conformation under these solution conditions. Furthermore, peptides 1–95 and

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                1                   2
acA S Q K R P S Q R H G S K Y L A T A S T M D H A R
        3                   4                   5
  H G F L P R H R D T G I L D S I G R F F S S D R G
                6                   7
  A P K R G S G K D H A A R T T H Y G S L P Q K S H
        8                   9                   0
  G R P Q D E N P V V H F F K N I V T P R T P P P S
                1                   2
  Q G K G R G T V L S R F S W G A E G Q K P G F G Y
        3                   4                   5
  G G R A A D Y K S A H K G L K G A D A Q G T L S R
                6
  L F K L G G R D S R S G S P M A R R
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FIGURE 5: Amino acid sequence of myelin basic protein of rabbit. Hydrophobic sequences, characterized by a "hydropathy" profile made according to Kyte & Doolittle (1982), are underlined, i.e., 15–21, 37–45, 84–92 (which contains the centrally located Phe-Phe sequence), 106–112, and 148–154. Ac denotes that the N-terminal alanine residue is acetylated. See also Weise (1985) for analyses of hydropathy profiles of myelin proteins.

96–168 exhibited different conformational adaptability as promoted by increasing concentrations of TFE.

Location of the sequences of the MBP that may be involved in the formation of β-structure and α-helix can be tentatively suggested on the basis of the data in Table I together with considerations of hydrophobicity (Figure 5) and the results of secondary structure prediction (Martenson, 1981) made according to Chou & Fasman (1978). In purely aqueous solution, it would be expected that the most highly hydrophobic sequences would associate and that only these would be capable of forming stable hydrogen-bonded β-structures. The hydrophobic peaks of MBP would correspond to sequences 15–21, 37–45, 84–92, 106–112, and 148–154 (Figure 5). All of these hydrophobic sequences lie within or contain sequences of predicted β-structure; sequences 15–21, 84–92, and 148–154, however, can alternatively be predicted as α-helical (Martenson, 1981).

1–95 Sequence. The β-structure observed in fragment 1–95 in the absence of TFE can be accounted for by a three-stranded sheet of 16–18 residues formed by antiparallel association of about 50% of the hydrophobic residues in sequences 15–21, 37–45, and 84–92. At TFE concentrations between 10% and 30%, a β-strand → α-helix transition could have occurred within sequence 15–21 so that it became part of an ~20-residue α-helix that formed in this solvent between residues 7 and 29. The ~10 additional helix residues formed within the transitions between 30% and 75% TFE were probably in sequences 58–63 and 70–75, since these are the longest remaining sequences lacking in numerous residues that hinder helix formation and propagation. Most of the residues of β-structure that formed between 20% and 75% TFE can be accounted for by an extension of the remaining two-stranded sheet to include Thr^{35} and Pro^{94} (see Figure 5). Electrostatic interactions between the side chains of Asp^{34} and Arg^{95} and between the side chain of Arg^{33} and the free carboxylate of Arg^{95} would be possible, as well as hydrogen bonding between the main-chain C=O of Thr^{35} and the side-chain OH of Thr^{93}. These interactions could offset the potential destabilization of β-structure resulting from the incorporation of Pro^{94} into the sheet.

It is noteworthy that the ~20 residues that became helical between 75% and 92% TFE must have arisen from the β-structure. The β-structure conformation stable in 75% TFE could have been altered by a qualitative change in the structure of the remaining aqueous solvent surrounding the residues in 92% TFE. Similarly, β-structure might constitute an obligate

intermediate in the formation of the α-helical patterns (including residues 84–93) that evolve in the presence of high concentrations of apolar groups (Mendz et al., 1984). The extent of β-structure remaining in 92% TFE (~5 residues) is uncertain at this low percent of β-structure.

96–168 Sequence. The β-structure observed in peptide 96–168 in the absence of TFE can be accounted for completely by a two-stranded sheet formed by antiparallel association of most of the two hydrophobic sequences, 106–112 and 148–154. In the presence of TFE up to 75%, this sheet could have extended inclusively to residues 113, 114, 146, and 147. The single transition of unordered conformations to α-helix, which involved up to ~10 residues at 75% TFE, probably occurred within sequence 129–140. In this 12-residue sequence, there are 7 "helix-forming" residues (3 Ala, 3 Lys, and 1 Leu), and the helix dipole (Hol et al., 1978) would be neutralized by the Asp in the first turn of the helix and the Lys at its C terminus. Between 75% and 92% TFE, approximately 18 additional residudes adopted the α-helical conformation at the expense of roughly an equivalent number of β-structure residues, a finding that can be explained by a transition of both β-strands to α-helices. As in peptide 1–95, there is a small amount of apparent β-structure that cannot be readily assigned.

Figure 4 and Table I show little change in the number of residues with unordered conformations above 50% TFE, suggesting that by this percent of solvent essentially all of the oligopeptide sequences with the potential for adopting ordered structures had done so. Examination of the sequences not assignable to either α-helix or β-structure at 50% TFE showed that the refractoriness was probably due to the presence of a relatively large number of residues that have a strong tendency to avoid α-helix or β-conformations. Indeed, half of the residues in the proposed 42–50% refractory, nonordered sequences of both peptides consisted of Pro, Gly, Ser, and Asp.

Judging from the data presented in Table I, residues in β-turns must have been relatively few. The very small contribution made by the model β-turn CD spectrum to the best-fitting CD spectra precludes an estimation of a definite number of β-turns; at most, there might be two in each peptide. These results are in contrast to certain predictions of β-turns in the secondary structure of the protein (Martenson, 1981). The low number of β-turns found experimentally can be reconciled with the predictions because of the sizable lengths of most of the hydrophilic segments that separate the β-strands and α-helices (Figure 5). These segments, as well as the hydrophilic C-terminal sequence, contain numerous residues with small or no side chains and would be highly flexible at several loci. Consequently, any chain reversals that would occur in these relatively long sequences as a result of β-sheet formation or helix–helix interactions elsewhere need not be confined to four residues such as those of β-turns [e.g., see Mattice & Scheraga (1985)]. It also should be noted that our CD analysis did not take into account the possible minor contribution of β-turns giving a β-structure-like (class A) or an α-helix-like (class C) spectrum (see Appendix for further comment).

When compared at 0% and 92% TFE, the two halves of the MBP behaved conformationally in an approximately additive fashion with regard to the intact protein. This was not unexpected, since the protein was cleaved at a bond (Pro-Arg/Thr-Pro-Pro-Pro) which does not involve formation or enlargement of β-structure or α-helical conformation, and since long-range interactions in the protein appear to be minimal (Krigbaum & Hsu, 1975; Martenson, 1978; Mendz et al., 1983). The β-sheet of the oligopeptides, however, need not have the same as those of the intact protein, since there are a variety of ways in which several β-strands can be ordered in a β-sheet.

Table II: Standard CD Spectra of Various Peptide Conformations[a]

wavelength (nm)	molar residue ellipticity					
	1	3	5	6	7	8
250	0	−50	0	0	0	0
248	−300	−200	0	0	0	0
246	−600	−500	0	0	0	0
244	−1 000	−800	0	0	0	0
242	−1 500	−1 200	0	0	0	0
240	−3 000	−2 100	200	−500	0	0
238	−5 000	−3 000	400	−1 000	0	0
236	−8 000	−4 300	550	−1 800	0	0
234	−11 300	−5 700	700	−2 500	0	−100
232	−15 400	−7 700	800	−2 700	0	−250
230	−20 000	−10 300	700	−2 900	0	−850
228	−25 000	−14 000	200	−3 100	50	−1 450
226	−28 500	−17 800	−1 410	−2 900	80	−2 000
224	−30 500	−18 900	−4 350	−1 900	200	−2 900
222	−31 500	−18 850	−7 900	−500	800	−3 800
220	−31 000	−18 100	−10 650	1 600	1 590	−4 800
218	−29 500	−16 900	−12 150	6 700	2 180	−6 200
216	−28 600	−15 900	−12 900	13 600	2 200	−7 500
214	−26 500	−15 300	−12 000	19 400	1 200	−8 100
212	−27 500	−15 000	−10 710	25 400	0	−8 600
210	−28 300	−15 200	−9 100	29 500	−2 580	−8 600
208	−29 100	−15 800	−6 450	31 000	−5 700	−8 400
206	−27 100	−13 300	−3 000	27 500	−9 400	−8 550
204	−18 100	−8 200	2 250	22 500	−14 000	−9 900
202	−4 300	−1 700	6 000	17 000	−19 200	−13 500
200	10 000	7 900	9 900	10 000	−22 300	−12 000
198	40 500	15 700	15 600	0	−24 400	−5 000
196	56 000	24 200	20 400	−7 500	−24 000	−1 600
194	68 000	29 800	21 000	−15 800	−21 600	−600
192	75 000	32 200	20 400	−21 100	−19 300	−200
190	68 000	28 100	16 650	−23 000	−16 300	−100
188	54 800	17 900	8 100	−21 600	−11 700	0
186	42 000	11 300	3 000	−19 000	−7 000	0
185	35 000	930	900	−17 500	−4 500	0

[a] Spectrum 1, α-H_L, is the spectrum of a long α-helix derived from both proteins and polypeptide models. Spectrum 2 (not shown) is the spectrum of a short α-helix (n = 5) derived from protein analysis by Chang et al. (1978). Spectrum 3, α-Hs(pp), is the spectrum of a short α-helix derived from the spectrum of poly(L-lysine) in a complex between the polypeptide and heparin, 1:1 by charge (A. L. Stone, unpublished results). Spectrum 4 (not shown) is the spectrum of a β-structure the intensity of which corresponds with that derived from proteins (Chang et al., 1978). Spectrum 5, β-P(pp), is the spectrum of a β-structure the intensity of which corresponds with that of polypeptides [in particular, that derived from poly(L-lysyltyrosine) in a complex between the copolypeptide and heparin, 1:1 by charge (Stone & Epstein, 1977), and from the homopolypeptide of lysine (Timasheff & Gorbunoff, 1967)]. Spectrum 6, β-t, is the theoretical class B spectrum of a β-turn conformation of peptides taken from Woody (1974). Spectrum 7 is the spectrum of an extended, nonordered polypeptide (ENOS) of poly(L-lysine) which was widely representative of polypeptides including various copolymers of lysine. Spectrum 8 is the spectrum of a partially collapsed, nonordered polypeptide (CNOS) structure obtained from the reaction between poly(L-lysine) and an acidic bacterial polysaccharide (AFP) (Stone & Smith, 1982) where the stoichiometry was 1:1 by charge.

Mattice & Robinson (1981) have previously calculated that sequences 10–25 and 128–155 of MBP are those that have a high probability for helix formation in the presence of sodium dodecyl sulfate. According to our interpretation of the present data, the above N-terminal sequence and part of the C-terminal sequence (i.e., residues 129–140) also form α-helices at relatively low concentrations (~30%) of TFE. In fact, a helix in sequence 129–140 would correspond to the first of the two peaks in the above helix propagation probability profile shown for sequence 128–155. However, we interpret our data as showing that helix formation corresponding to the second

Table III: Best-Fit Analyses of Four Peptide Conformations Using Matrix 7 of Standard CD Curves

	% (±SD) of four conformations at TFE concn (%)						
	0	10	20	30	50	75	92
1–95 peptide							
α-H_L	0 (2)	0 (2)	15 (2)	21 (3)	28 (2)	33 (2)	54 (2)
β-P_{PP}	18 (7)	18 (8)	15 (10)	19 (12)	26 (9)	25 (11)	5 (8)
β-t	0.7 (3)	5 (3)	0 (4)	0 (5)	4 (4)	0 (5)	0 (3)
ENOS	82 (5)	78 (5)	69 (7)	59 (8)	42 (6)	42 (7)	41 (5)
96–168 peptide							
α-H_L	0 (1)	0.8 (1)	7 (1)	10 (1)	12 (1)	14 (1)	39 (2)
β-P_{PP}	23 (4)	30 (4)	33 (5)	32 (5)	34 (6)	35 (6)	8 (7)
β-t	4 (2)	2 (2)	7 (2)	6 (2)	5 (2)	7 (2)	0 (3)
ENOS	73 (3)	67 (3)	52 (3)	51 (3)	50 (4)	45 (4)	53 (5)
MBP[a]							
α-H_L	0.4 (2)						47 (2)
β-P_{PP}	17 (9)						6 (7)
β-t	0 (3)						0 (3)
ENOS	82 (5)						47 (4)

[a] 250–192 nm at 0% TFE and 250–190 nm at 92% TFE.

peak in the profile (i.e., residues 147–154) does not occur unless the concentration of TFE is greater than 75%, thereby leading to a transition from the β-structure in this sequence.

In contrast, by a comparison between the CD spectra of peptides 1–87 and 1–95 of MBP bound to micelles of dodecylphosphocholine, Mendz et al. (1984) found that a major helix-forming region existed in the hydrophobic sequence 84–93. Furthermore, binding to the micelles resulted in comparatively little helix formation in fragments 1–87 and 98–168 and essentially none in fragments 1–44 and 45–87. Comparable results had also been obtained by Keniry & Smith (1979, 1981) with bovine MBP peptides bound to phosphatidylserine vesicles. These results differ from those predicted by Mattice & Robinson (1981) and from our interpretation of the present results. The putative β-structure $\rightarrow$ α-helix transition that would allow residues 84–93 to be helical in TFE in the presence of sufficiently high concentrations of the apolar groups of the solvent further underscores the complexity of the physicochemical interactions that might influence the protein's conformation in situ.

Similar best-fit analyses are currently under way of MBP at the intermediate concentrations of TFE and of shorter peptides derived by cleavage of MBP within each of the larger segments. Such data should enable us to discuss more precisely the location of the putative conformationally adaptable sequences within the complete protein.

Appendix

Most of the standard CD spectra utilized in the 10 final tests of the best-fit analyses are listed in Table II along with descriptions of the various spectra. Spectrum 8 resembles that proposed by Tiffany & Krim (1969) for the partially collapsed nonordered structure in that the newly formed peak is at ~210 nm. Matrix 7 gave the best-fitting analyses when compared with the experimental spectra. Table III summarizes these results, which are expressed in percent of each of the four peptide conformations of the best-fit composite, accompanied by the standard error of the percent. Data are also shown for rabbit MBP (at 0.21 mg/mL). The wavelength range was 250–192 nm in 0% TFE and 250–190 nm in 92% TFE because the experimental errors increased sharply at 188 nm and below.

Matrix 7 was composed of spectra 1, 5, 6, and 7 (Table II); matrix 8 = 3, 5, 6, and 7; matrix 9 = 1, 5, 6, and 8. Matrix 8 and matrix 9 were employed to gain further insight into the conformational aspects of the oligopeptides (see text).

The theoretical CD spectra of the various conformations of the major types of β-turns were computed by Woody (1974) and found to commonly yield a similar type of spectral display called the class B spectrum. To a lesser extent, three other patterns (classes A, C, and D) might occur. Furthermore, although experimental model peptides in the β-turn conformation were also found to commonly exhibit a class B type CD spectrum, these can be shifted to lower wavelengths relative to the theoretical curve [e.g., see Gierasch et al. (1981)]. Recent studies of linear model peptides in β-turn conformation have also shown that CD contributions other than that of class B type might be expected in the presence of a number of proline-containing β-turns (Hollősi et al., 1985). In the absence of adequate information about conformations of the possible β-turns, the computed class B spectrum of a β-turn of Woody (1974) was employed in all matrices; this type of spectrum would be expected to approximate that of a majority of the β-turn structures that might occur in the MBP-derived oligopeptides. The effect of this assumption on the estimate of the percent of β-turn and the best-fit composite has been considered. β-Turns that have class B spectra might be slightly underestimated, tending to increase the error in the computed β-structure component. If present, β-turns having CD of the spectral class A or C would also be underestimated, causing instead a slight overestimation of β-structure or α-helix, respectively. Finally, the possible Pro-Ser-containing β-turn in peptide 1–95 would not have been counted; if present, its relatively small CD component in the experimental spectrum (Hollősi et al., 1985) would have contributed to the deviations from the best-fit composite spectrum. A thorough review of the CD of many possible experimental β-turn conformations can be obtained from Smith & Pease (1980).

The results in Table III point out that the actual number of β-turns within the oligopeptides is probably small, which would tend to make such errors insignificant among the total errors. However, a more quantitative assessment of these possible errors might be revealed by further studies utilizing various types of β-turn spectra derived from known experimental β-turns.

Registry No. CF_3CH_2OH, 75-89-8.

References

Anthony, J. S., & Moscarello, M. A. (1971) *Biochim. Biophys. Acta 243*, 429–433.

Block, R. E., Brady, A. H., & Joffe, S. (1973) *Biochem. Biophys. Res. Commun. 54*, 1595–1602.

Brahms, S., & Brahms, J. (1980) *J. Mol. Biol. 138*, 149–178.

Brahms, S., Brahms, J., Spach, G., & Brack, A. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3208–3212.

Carnegie, P. R., & Moore, W. J. (1980) in *Proteins of the Nervous System* (Bradshaw, R. A., & Schneider, D. M., Eds.) 2nd ed., pp 119–143, Raven Press, New York.
Carver, J. P., Schecter, E., & Blout, E. R. (1966) *J. Am. Chem. Soc. 88*, 2550–2561.
Chang, C. T., Wu, C.-S. C., & Yang, J. T. (1978) *Anal. Biochem. 91*, 13–31.
Chen, Y.-H., Yang, J. T., & Martinez, H. M. (1972) *Biochemistry 11*, 4120–4127.
Chen, Y.-H., Yang, J. T., & Chan, K. H. (1974) *Biochemistry 13*, 3350–3357.
Chou, P. Y., & Fasman, G. D. (1978) *Adv. Enzymol. Relat. Areas Mol. Biol. 45*, 45–148.
Davidson, B., & Fasman, G. D. (1967) *Biochemistry 6*, 1616–1629.
Deibler, G. E., & Martenson, R. E. (1973) *J. Biol. Chem. 248*, 2392–2396.
Deibler, G. E., Martenson, R. E., Kramer, A. J., Kies, M. W., & Miyamoto, E. (1975) *J. Biol. Chem. 250*, 7931–7938.
Deibler, G. E., Krutzsch, H. C., & Martenson, R. E. (1985) *J. Biol. Chem. 260*, 472–474.
Eagland, D. (1975) *Water: Compr. Treatise 4*, 497–510.
Gierasch, L. M., Deber, C. M., Madison, V., Nir, C.-H., & Blout, E. R. (1981) *Biochemistry 20*, 4730–4738.
Golds, E. E., & Braun, P. E. (1976) *J. Biol. Chem. 251*, 4729–4735.
Greenfield, N., Davidson, B., & Fasman, G. D. (1967) *Biochemistry 6*, 1630–1637.
Hol, W. G. J., van Duijnen, P. T., & Berendsen, H. J. C. (1978) *Nature (London) 273*, 443–446.
Hollősi, M., Kawai, M., & Fasman, G. D. (1985) *Biopolymers 24*, 211–242.
Holzworth, G., & Doty, P. (1965) *J. Am. Chem. Soc. 87*, 218–228.
Keniry, M. A., & Smith, R. (1979) *Biochim. Biophys. Acta 578*, 381–391.
Keniry, M. A., & Smith, R. (1981) *Biochim. Biophys. Acta 668*, 107–118.
Kies, M. W., Murphy, J. B., & Alvord, E. C., Jr. (1961) in *Chemical Pathology of the Nervous System* (Folch-Pi, J., Ed.) pp 197–206, Pergamon Press, London.
Knott, G. (1979) *Comput. Programs Biomed. 10*, 271–280.
Kornguth, S. E., & Perrin, J. H. (1971) *J. Neurochem. 18*, 983–988.
Krigbaum, W. R., & Hsu, T. S. (1975) *Biochemistry 14*, 2542–2546.
Kyte, J., & Doolittle, R. F. (1982) *J. Mol. Biol. 157*, 105–132.
Laatsch, R. H., Kies, M. W., Gordon, S., & Alvord, E. C., Jr. (1962) *J. Exp. Med. 115*, 777–788.
Law, M. J., Martenson, R. E., & Deibler, G. E. (1984) *J. Neurochem. 42*, 559–568.
Liebes, L. F., Zand, R., & Phillips, W. D. (1975) *Biochim. Biophys. Acta 405*, 27–39.
Martenson, R. E. (1978) *J. Biol. Chem. 253*, 8887–8893.
Martenson, R. E. (1981) *J. Neurochem. 36*, 1543–1560.
Martenson, R. E., Lüthy, V., & Deibler, G. E. (1981) *J. Neurochem. 36*, 58–68.
Mattice, W. L., & Robinson, R. M. (1981) *Biopolymers 20*, 1421–1434.
Mattice, W. L., & Scheraga, H. A. (1985) *Biopolymers 24*, 565–579.
Mendz, G. L., & Moore, W. J. (1983) *Biochim. Biophys. Acta 748*, 176–183.
Mendz, G. L., Moore, W. J., & Martenson, R. E. (1983) *Biochim. Biophys. Acta 742*, 215–223.
Mendz, G. L., Moore, W. J., Brown, L. R., & Martenson, R. E. (1984) *Biochemistry 23*, 6041–6046.
Moffit, W. (1956) *Proc. Natl. Acad. Sci. U.S.A. 42*, 736–746.
Omlin, F. X., Webster, H. deF., Palkovits, C. G., & Cohen, S. R. (1982) *J. Cell Biol. 95*, 242–248.
Poduslo, J. F., & Braun, P. E. (1975) *J. Biol. Chem. 250*, 1099–1105.
Quadrifoglio, R., & Urry, D. W. (1968) *J. Am. Chem. Soc. 90*, 2755–2760.
Saxena, V. P., & Wetlaufer, D. B. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 969–972.
Sedzik, J., Blaurock, A. E., & Höchli, M. (1984) *J. Mol. Biol. 174*, 385–409.
Smith, F. A., & Pease, L. G. (1980) *CRC Crit. Rev. Biochem. 8*, 315–399.
Stone, A. L., & Epstein, P. (1977) *Biochim. Biophys. Acta 497*, 298–306.
Stone, A. L., & Smith, E. J. (1982) XIth International Carbohydrate Symposium, Vancouver, British Columbia, Canada.
Swann, R. W., & Li, C. H. (1979) *Int. J. Pept. Protein Res. 14*, 495–503.
Tiffany, M. L., & Krimm, S. (1969) *Biopolymers 8*, 347–359.
Timasheff, S. N., & Gorbunoff, M. J. (1967) *Annu. Rev. Biochem. 36*, 13–20.
Urry, D. W., Long, M. M., Ohnishi, T., & Jacobs, M. (1974) *Biochem. Biophys. Res. Commun. 61*, 1427–1433.
Weise, M. J. (1985) *J. Neurochem. 44*, 163–170.
Woody, R. W. (1974) in *Peptides, Polypeptides and Proteins* (Blout, E. R., Bovey, F. A., Goodman, M., & Lotan, N., Eds.) pp 338–348, Wiley, New York.
Yang, J. T., Bewley, T. A., Chen, G. C., & Li, C. H. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3235–3238.