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Conformation of bovine myelin basic protein purified with bound lipids

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Abstract The basic protein of myelin (called MBP) is an extrinsic protein of the myelin membrane. Its structure and function are still unknown. MBP has been extensively studied in its water-soluble form, but it is also known in a detergent-soluble form, which is purified with endogenous myelin lipids and should correspond to the native form of the protein in the membrane. In order to acquire insight into the structure of MBP, we have carried out circular dichroism (CD) experiments on the protein both in the lipid-free and in the lipid-bound form. Our data clearly show that lipid-free MBP is mainly disordered with only a small amount having α-helix and β-sheet motifs. On the other hand, the lipid-bound form of MBP appears to have a consistent amount of ordered secondary structure. Theoretical predictions, made using different computational methods, substantially confirm the tendency of the protein to assume an ordered secondary structure in accordance with our CD results.

Key words Myelin basic protein · Circular dichroism · Computational predictive methods · Protein structure · Lipid-protein interaction

Abbreviations *CCA* Convex constraint analysis · *CHAPS* 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate · *LB-MBP* Lipid-bound MBP · *LF-MBP* Lipid-free MBP · *MBP* Myelin basic protein · *SDS* Sodium dodecyl sulfate

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Introduction

Myelin is the multilamellar membranous sheath surrounding nerve axons and myelin basic protein (MBP) is a major protein of this membrane both in the central (CNS) and peripheral nervous systems (PNS) (Martenson 1992; Smith 1992). MBP has been extensively studied as the autoantigen responsible for experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis (Martenson 1992), and as the main agent in the formation and compaction of CNS myelin (Riccio et al. 1986; Readhead et al. 1987). Attempts to determine MBP structure in compact myelin have failed. Spectroscopic studies carried out on MBP purified in aqueous solution have shown that the protein probably has a substantially disordered conformation, which is however prone to changes in the presence of detergent and lipids (Martenson 1992). On these grounds, great interest has been devoted to understanding how the water-soluble, lipid-free MBP (LF-MBP) interacts with lipids in the membrane and how this interaction may influence MBP structure. Using a different approach to the problem, MBP has been extracted from the myelin membrane with detergents and purified with bound lipids (Riccio et al. 1984, 1990, 1994). Lipid-bound MBP (LB-MBP) was found to differ in several functional aspects from the corresponding lipid-free form of the protein (Riccio et al. 1986; Bobba et al. 1991; Lolli et al. 1993; Massacesi et al. 1993; Liuzzi et al. 1996; Vergelli et al. 1997; Mazzanti et al. 1998), but a deep investigation of its structure has long been difficult because of the presence of detergent, lipids and protease inhibitors in the sample mixture. Using circular dichroism (CD) on MBP preparations obtained at low detergent concentrations and in the absence of interfering additives, in this paper we demonstrate that the MBP copurified with all the myelin lipids possesses a more ordered structure than the lipid-free form of the protein. This result is in good agreement with theoretical predictions and suggests that such a structure is a native property of MBP in the myelin sheath.

Materials and methods

Preparation of myelin

Myelin was prepared from bovine brain white matter using two cycles of sucrose gradient plus osmotic shock. Material floating on 0.8 M sucrose after the second gradient centrifugation was washed twice and stored at –70 °C (Riccio et al. 1994).

Purification of LF-MBP

MBP was purified in the lipid-free, denatured form according to Deibler et al. (1972, 1984).

Purification of LB-MBP

MBP was purified in the lipid-bound, native-like form using the zwitterionic detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) (Boehringer, Mannheim) according to the procedure previously described (Riccio et al. 1994), but in the absence of additives such as protease inhibitors, and in one step only. Briefly, following a pretreatment of myelin with 0.5 M NaCl and 20 mM Hepes, pH 7.4, LB-MBP was extracted from the myelin residue using 2% CHAPS and 20 mM Hepes, pH 7.4, and applied to a hydroxyapatite column equilibrated with 1% CHAPS and 20 mM Hepes, pH 7.4. LB-MBP was collected from the non-adsorbed, pass-through fractions, lyophilized and stored in a deep freezer at –70 °C until use. The minimum lipid content of MBP after dialysis was 1.5 mg phospholipids/mg protein. As assessed by densitometric analysis of TLC plates (3 experiments), the relative percentage of lipids bound to MBP were the following: cholesterol 26 ± 2, cerebrosides 6.5 ± 2, sulfatides 8.0 ± 8.0; phosphatidylethanolamine 32.6 ± 9.1; phosphatidyl inositol 5.2 ± 0.8; phosphatidylserine 4.3 ± 1.3; phosphatidylcholine 15.60 ± 0.04; sphingomyelin 3.50 ± 0.04.

Purification of myelin lipids

Myelin lipids were extracted from bovine brain with 19 volumes of chloroform/methanol (2 : 1), as described in the procedure for purification of LF-MBP. Proteins were separated using the procedure of Folch et al. (1957). The lipidic phase was dried under nitrogen and stored at –70 °C.

Absorption measurements

Absorption spectra were obtained using a Jasco 7850 UV-VIS spectrophotometer. The protein concentration was evaluated assuming a molar extinction coefficient ε =10 300 M^{-1} cm^{-1} at 276.4 nm (Liebes et al. 1975). In the case of LB-MBP, where scattering was present, the absorption spectra were corrected in accord to Cavatorta et al. (1986).

Fluorescence measurements

Fluorescence measurements were performed using a Perkin-Elmer LS50 spectrophotofluorimeter. The lipid-free protein was dissolved in 20 mM Hepes buffer, pH 7, and the lipid-bound protein in the same buffer plus detergent, immediately before use. The excitation wavelength was kept at 295 nm, where only the single tryptophan residue Trp115 absorbs. The absorbance of the samples at the same wavelength never exceeded the value of 0.03, in order to avoid inner filter effects.

Circular dichroism measurements

CD experiments were carried out using a Jasco J715 spectropolarimeter, calibrated with ammonium *d*-10-camphorsulfonic acid. The measurements were performed in the far-UV spectral region, where also the detergent absorbs. To minimize the inner filter effect caused by lipids and detergent, the spectra were obtained using a cell of 0.01 cm optical pathlength, at a protein concentration of 2.5×10^{-5} M. In these conditions the absorbance of the samples in the presence of detergent was about 1 at 190 nm.

In order to reduce scattering artifacts, the cell was always placed immediately before the photomultiplyer. However, moving the cell along the optical path in the cell compartment did not cause any detectable variation in the CD spectra.

The relative amounts of ordered secondary structure in the proteins were evaluated by analyzing the spectra with the convex constraint analysis (CCA) method, developed by Perczel et al. (1991, 1992).

Theoretical predictions

The secondary structure of MBP was predicted with 10 of the most used prediction tools by means of different web servers. They are: the Gibrat method (Gibrat et al. 1987), the Levin method (Levin et al. 1986), the double prediction method (DPM) (Deleage and Roux 1987), the self-optimized prediction method from alignments (SOPMA) (Geourjon and Deleage 1995), the PHD predict protein (Rost and Sander 1994), the binary word method, combined with the GOR and the neural network method (BW-MGOR) (Kawabata and Doi 1997) the quadratic-logistic (QL) (DiFrancesco et al. 1995), the neural network (NN) method (Kneller et al. 1990), the Garnier-Osguthorpe-Robson method, version IV (GOR-IV) (Garnier et al. 1996) and the predator method (Frishman and Argos 1995). The sequence of bovine MBP was retrieved from the Swiss-Prot databank (accession number: P02687).

Results and discussion

The fluorescence spectra of LF-MBP in buffer, LF-MBP in 0.5% CHAPS and LB-MBP in 0.5% CHAPS are shown in Fig. 1. The spectrum of LF-MBP in buffer (spectrum a) shows an emission maximum at 355 nm, indicating that the Trp115 residue is exposed to the solvent, in agreement with previous reports (Cavatorta et al. 1988). The addition of 0.5% CHAPS (spectrum b) causes an increase in fluorescence intensity and a small but detectable blue shift (1.5 nm) in the maximum wavelength. The interaction of LF-MBP with detergents has been extensively investigated (Smith 1992). In general, it has been suggested that LF-MBP interacts strongly with anionic detergents such as sodium dodecyl sulfate (SDS) (Smith and McDonald 1979; Burns et al. 1981; Burns and Campagnoni 1983) and less strongly with non-ionic detergents that can, in some cases, cause the formation of hexameric complexes of the protein (Mendz et al. 1984, 1990). The fluorescence behavior of LF-MBP with CHAPS suggests that the detergent micelles can bind to some sites of the protein, slightly protecting the Trp fluorescence from the quenching effect of the solvent.

The presence of the intrinsic lipids around MBP (LB-MBP, spectrum c) causes a further increase in the intensity of the fluorescence emission spectrum and a blue shift of 7.5 nm of the maximum. This effect could be explained by an increased shielding of the Trp residue from the solvent and provides evidence that the lipids are bound to the protein.

Figure 2 contains the CD spectra of LF-MBP in buffer, LF-MBP in 0.5% CHAPS and LB-MBP in 0.5% CHAPS, and the best fits of the experimental data obtained with the CCA method (Perczel et al. 1991, 1992). With this method the percentage of secondary structure has been estimated from the five pure component curves that can be attributed to the α-helix, antiparallel β-sheet, β-turn, random coil and "other" structures (Table 1).

In order to see if the lipids bound to the protein contribute to the CD spectra, we carried out a measurement on a suspension of lipid extracted from myelin with the detergent, at a concentration comparable to that present in the protein sample. In the wavelength range used for the secondary structure analysis (195–240 nm), the lipids did not show any detectable CD signal.

From the analysis performed on the CD spectra the changes in the amount of secondary structure corresponding to changes in the intensity of each pure component curve can be seen. In particular, the increase in the ordered α- and antiparallel β-structures on going from a polar (LF-MBP) to an apolar (LB-MBP) environment is quite evident. Such an increase corresponds to a loss of the random coil and, to a lesser extent, of the β-turn structures. The "other" type of contributions totally disappear in the LB-MBP spectrum. These results demonstrate that MBP assumes a more ordered conformation in the lipid-bound form. This is the first CD study carried out on MBP copurified with bound myelin lipids as a lipid-protein detergent complex. A very large set of experiments on the MBP conformation has been already carried out, but upon adding specific lipids to lipid-free MBP (for a review see Smith 1992). More recently, it has been shown that addition to MBP of lipids extracted from myelin can actually induce an increase in the secondary structure of MBP (Stuart 1996).

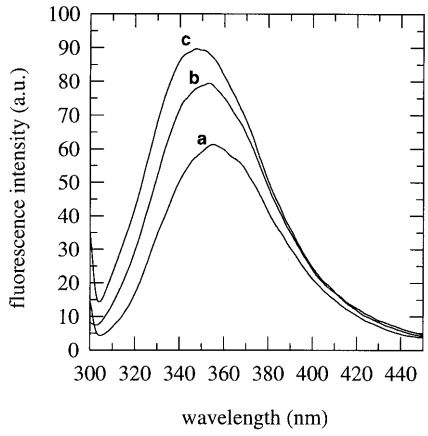

Fig. 1 Steady-state emission spectra of LF-MBP (*a*), LF-MBP with 0.5% CHAPS (*b*) and LB-MBP with 0.5% CHAPS (*c*) in 10 mM Hepes buffer, pH 8. The emission maximum wavelengths are respectively 355, 353.5 and 347.5 nm

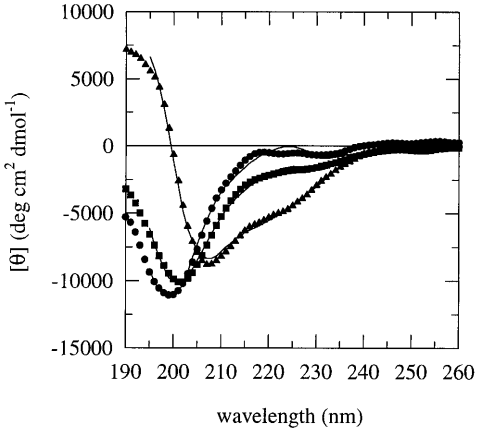

Fig. 2 CD spectra of LF-MBP (*circles*), LF-MBP with 0.5% CHAPS (*squares*) and LB-MBP with 0.5% CHAPS (*triangles*) in 10 mM Hepes buffer, pH 8, and the best fits (*solid line*) of the experimental data obtained with the CCA method (Perczel et al. 1991, 1992)

Table 1 Decomposition of MBP CD spectra by the CCA method

	α-Helix (%)	Antiparallel β-sheet (%)	β-Turn (%)	Random coil (%)	Other structure (%)
LF-MBP	5.1	6.0	35.9	52.7	0.3
LF-MBP + CHAPS	8.6	7.2	32.4	51.7	0.1
LB-MBP	23.8	20.3	27.9	28.0	0.0

Table 2 Theoretical prediction of secondary structure of MBP[a]

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                1       10        20        30        40        50        60
                |        |         |         |         |         |         |
MBP sequence    AAQKRPSQRSKYLASASTMDHARHGFLPRHRDTGILDSLGRFFGSDRGAPKRGSGKDGHH
Gibrat method   HHH....HHEEEEHHHHHHHHHHH.EE.......EEEEEEEEE...............EE
Levin method    ........H.HHH...HHHHHHHH..........HHH.H.H...................
DPM method      ..H........EHH.H..H.HHH...........EE.....E..................
SOPMA predict   E.......HHHHHHHHHHHHH............HHHHHHH....................
PHD             ............EEEE..HHHH.............HHHHHH...................
BW-MGOR         .........HHEHH...HHHHHHH...........HH..HHHE.................
QL              HH........HHH..HHHHHHHH............E......................HH
NN              ..........HEEE...HHHH..............H.HH..EE................H
GOR-IV          ........HHHHHHHH.HHHHHH..EE.......EE........................
PREDATOR        ............................................................

                                 I                    II

                        70        80        90       100       110       120
                         |         |         |         |         |         |
MBP sequence    AARTTHYGSLPQKAQGHRPQDENPVVHFFKNIVTPRTPPPSQGKGRGLSLSRFSWGAEGQ
Gibrat method   EEEEEEE.....HH..........HHHEEE................EEEEEEEEE.....
Levin method    HE....E....H............HHHHHHH.E..............EEEEE........
DPM method      HHH.....................EEEEEEEEEE...............EEEE.......
SOPMA predict   HHHHHHH.................HHHHHHHEE.............EEEEEE........
PHD             ...EE...................EEEEEE..................EEEEEEE.....
BW-MGOR         ........................EEEEEE.................EEE.HH.......
QL              HHHHHH.....HH............EEEEE.EE..............EEHEHHHH.H...
NN              H.......................HHHH....................EE.EE.......
GOR-IV          EEEEE...................EEEEEE................EEEEE.........
PREDATOR        .........................EEEE....................EEE........

                 III                        IV                      V

                       130       140       150       160
                         |         |         |         |
MBP sequence    KPGFGYGGRASDYKSAHKGLKGHDAQGTLSKIFKLGGRDSRSGSPMARR
Gibrat method   ....EEE..E..HHHHHHHHE..HHHHHHHHEEEE..........EEEE
Levin method    ..........HHHHHH.HH.....HHHHHHHEEE............EEE
DPM method      ............................EEEEE............HH..
SOPMA predict   ..........HHHHHH...........HHHHHH.............HHH
PHD             ...........................EHHHHHH...............
BW-MGOR         ....E.......HHH.HH..........HHHHEEE..............
QL              .........HHHHHHHHH.......H..HHHHEEE..........HHHH
NN              ..............HHHH.........HHHHHHHH..............
GOR-IV          ............HHHHHH.........HHHHHHHH..........EEE.
PREDATOR        ..........................HHHHHEEE...............

                             VI              VII
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[a] H = helix, E = extended (beta strand)

Following the CD experiments, the protein has also been analyzed using computational predictive methods in order to give support to the results obtained by spectroscopic techniques. Computational analysis has also been reported by Stoner (1984, 1990), Martenson (1986) and, recently, by Ridsdale et al. (1997). The different kinds of predictive methods have made it possible to identify seven potentially structured segments (Table 2). Segments I–VII are formed by residues 9–24 (I), 35–43 (II), 61–67 (III), 85–93 (IV), 107–115 (V), 131–139 (VI) and 146–155 (VII), respectively. The average flexibility of the protein, as evaluated by Bhaskaran and Ponnuswamy (1984), is high (data not shown), with all the peaks situated in correspondence with the loops between the seven segments. This result confirms the quality of the prediction.

It is very interesting to note that five of the seven structured segments correspond exactly to the five putative β-sheets proposed by Stoner's model (Stoner 1984, 1990). They are segments I, II, IV, V and VII. Segments IV and V are the only ones that are predicted strictly in the extended structure by almost all the predictive methods, and it can be concluded that they are the most stable in the different environments. Segment I is the most ambiguous segment, because the prediction shows a helix pattern at variance with the β-sheet structure presented in the Stoner model.

Segments III and VI are in the region of the two α-helices proposed in the same model. The prediction confirms this fact, so segments III and VI can be considered as α-helices. The hydrophobicity plot (Kyte and Doolittle 1982; Eisenberg et al. 1984) and the calculation of the residue accessibility (Janin 1979) indicate (data not shown) that these two segments could be exposed on the surface of the molecule.

The prediction gives the potential structure of the molecule. It is plausible that a lipid environment can stabilize the ordered conformation of extrinsic membrane proteins, so that the prediction could give the structure of the protein in the presence of its intrinsic lipids (LB-MBP). Lipids could shield the molecular structure also, stabilizing it in an unfavorable environment.

When this theoretical analysis is compared with the results obtained from the CD measurements, we can put forward the following hypothesis:

1. In aqueous solution the MBP is substantially unordered, perhaps with a low amount of α-helix (5–9%) and β-sheet (6–10%) that could correspond to segments III and VI for helix and segments IV and V for β-sheet structures.
2. In the presence of intrinsic lipids the protein assumes a more ordered structure, showing four β-sheet regions (segments II, IV, V and VII) and two α-helix regions (segments III and VI). We suppose segment I is an α-helix, in agreement with the theoretical prediction, because in this case the amounts of α-helix (18.9%) and β-sheet (21.3%) fit well with the results of the CD analysis (23.8% and 20.3%, respectively). However, the possibility that segment I could have a β-sheet structure, as predicted by Stoner and Martenson, cannot be ruled out at this time. The prediction of β-turns, obtained with two different methods (Chou and Fasman 1978; Levitt 1978), gives a value of 27.2% which corresponds strictly with that obtained by CD (27.9%).

All the structural information collected will be used in the near future for the construction of theoretical models for the three-dimensional protein structure. By means of molecular dynamic techniques, we will try to determine the most stable conformation in the different environments.

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