Role of the MBP protein in myelin formation and degradation in the brain

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Abstract

The compact myelin sheath functions as an insulator for efficient conduction of nerve impulses. The formation of myelin sheaths around the axons of the most actively functioning neurons continues not only at the stage of brain development, but also in the process of learning and acquiring certain skills. Pathological or age-related disruption in myelin results in nerve conduction failure and neurodegeneration. Myelin Basic Protein (MBP) is the main constituent of the myelin sheath, representing about 30\% of the total myelin proteins in the central nervous system. Deletion in the MBP coding gene in mutant mice causes a severe neurological phenotype associated with rapid death of newborns. In this review, we discuss the current understanding of the role of the MBP protein in the formation of compact myelin and in neurodegeneration associated with demyelination.

Keywords: myelin, MBP, multiple sclerosis, oligodendrocyte, axon, mammalian brain, amyloid

Introduction

Myelin is a specialized sheath that forms around the axons of many actively working neurons and functions as an insulator in conducting nerve impulses between the body of the neuron and its target (Hartline and Colman, 2007). Compact myelin sheaths are formed by specialized glial cells — oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS). They are wrapped around the axon in a spiral fashion (Monje, 2018). Myelin of the central and peripheral nervous systems are functionally and structurally similar. The main difference between them is that in the CNS, one myelinating Schwann cell envelops one axon by itself; while in the CNS a single oligodendrocyte forms numerous flattened processes that can simultaneously myelinate different axons, while the bodies of the glial cells do not participate in myelination. Myelin of nerve fiber is not presented as a continuous sheath along the axon; in contrast, it forms separate segments or "internodes". Each myelinated segment of axon is flanked by narrow portions uncovered by myelin, called nodes of Ranvier, which are critical to the nerve functioning (Nave and Werner, 2014) (Fig. 1A). Myelinated nerve fibers, along with other glial cells and blood vessels, form the so-called white matter (WM) of the brain.

Myelin facilitates the conduction of nerve impulses by serving as an electrical insulator. Unmyelinated fibers have voltage-gated sodium channels along the entire length of the membrane, so the action potential travels as local circuits of ion current continuously (de Col, Messlinger and Carr, 2008). In contrast, in myelinated axons, the axolemma of nodes of Ranvier contains a high density of Na\(^+\)-channels against a relatively small number of channels on the membrane.
of internodes. When the membrane at the node is excited, the local currents flow further to the axolemma in the internodes; here, due to the insulating properties of the myelin sheath, the impulse does not attenuate, but “jumps” to the membrane at the next node. Thus, high-resistance sheath contributes to the preservation of the action potential in the nerve fiber, and the excess of Na+-channels in the membrane of Ranvier’s nodes amplifies the transmitted signal each time, which leads to an increase in the speed of local circuit spreading (Huxle and Stämpfli, 1949). Active excitation of the axonal membrane jumps from one node to the next; this causes a fast saltatory propagation of the impulse (from the Latin saltare — “to jump”), in contrast to unmyelinated nerve fibers, where action potentials are transferred in orders of magnitude slower (Ritchie, 1982).

According to transmission electron microscopy (TEM) data, mature myelin contains “compact” and “non-compact” areas (Stadelmann, Timmler, Barrantes-Freer and Simons, 2019). Non-compact myelin is present in the form of cytoplasm-filled membrane cavities in the outer and inner layers of the myelin sheaths as well as in the paranodal loops — specialized structures located on lateral margins of the myelin segments (Peters, 1960). These compartments perform a signaling and trophic function, supplying energy substrates from the myelin sheath to the axon, including lactate and pyruvate (Fünfschilling et al., 2012). Compact myelin is characterized by a unique molecular composition providing its insulating properties. The dry mass of myelin contains a high proportion of lipid (70–85 %), whereas the protein content is very low — 15–30 %, of which myelin basic protein (MBP) is one of the two most abundant proteins (Norton and Cammer, 1984). In the CNS, MBP constitute about 30 % of total myelin protein by weight, while in the PNS its quantity is minimal, and its deficiency is compensated for by adhesive PNS-specific P0 protein (Martini et al., 1995). MBP is an essential

![Fig. 1.](image-url)

(A) The diagram showing compact myelin structure formed by the oligodendrocyte processes (blue) that are wrapped around the axon (purple). (B) Compact myelin is represented by a series of alternating major dense (MDL) and intraperiod (IPL) lines. (C) Proposed model of MBP binding to lipid bilayers within myelin composition. A cross section of a flattened process of an oligodendrocyte in the area of compact myelin formation is shown. The N and C-terminal sequences of MBP (red) bind to lipids (blue) of the opposite process membranes of the oligodendrocyte. (D) Amyloid model of MBP protein folding within myelin sheath. According to this model MBP molecules not only bind opposite membranes, but also form amyloid fibrils inside the process of the oligodendrocyte.
structural component of mature compact myelin, since disruptions in its production cause severe demyelination of the CNS. For instance, a mouse shiverer mutation, which results from a large deletion in the MBP gene, impairs the structural integrity of the myelin sheath and causes the classic symptoms of demyelination, accompanied by whole body tremors, seizures, progressive ataxia, hindlimb paresis and early death (Bourre et al., 1980; Jacque, Delassalle, Raoul and Baumann, 1983; Fitzner et al., 2006). Similar myelin deficits also result from a rat Long Evans shaker (les) mutation upon the insertion of a 5.7 kb endogenous retrotransposon in the non-coding region (intron 3) of the MBP gene, which alters the normal splicing dynamics of MBP mRNA and decreases its expression level (O'Connor et al., 1999).

In TEM, a compact myelin sheath is visualized as a series of alternating dark and pale lines, separated by unstained lipid-rich layers (Fig. 1B) (Stoeckenius, 1959; Peters, 1960). A pale line, or intraperiod, corresponds to closely apposing extracellular surfaces of the oligodendrocyte plasma membrane. The dark, or major dense line (MDL) represents the inner protein-rich layer between the cytoplasmic sides of oligodendroglia membranes. MBP is located between cytoplasmic membrane faces in MDL, stabilizing the multilayer myelin structure through its anchoring to opposite membrane sides with N- and C-termini (Fig. 1C) (Harauz and Libich, 2009). MBP is assumed to be retained within this layer by electrostatic forces with membrane lipids. This molecule is characterized by a high positive net charge caused by its large number of charged residues throughout the protein's sequence, mostly arginine and lysine (Boggs et al., 2004). Its high overall positive charge and low hydrophobicity, which maximize intramolecular electrostatic repulsion, causes an internal conformational disorder of protein in solution, which allows it to interact with the negatively charged phospholipid-rich cytoplasmic surface of myelin membranes (Raasakka et al., 2017). Thus, the adhesive role of MBP is supposed to be fundamental in myelin assembly.

Heterogeneity of MBP isoforms

Human MBP protein is produced by the Golli-MBP gene complex, which localizes on Chromosome 18q (de Ferra et al., 1985), consists of 11 exons and has two transcription initiation sites (Campagnoni et al., 1993). This gene produces two subfamilies of proteins — the “classical” myelin-specific MBP isoforms which are present in myelinating oligodendrocytes in the CNS and Schwann cells in PNS; and “Golli” MBP variants (the “Golli” prefix is an abbreviation for gene expressed in the oligodendrocyte lineage) that are found, besides the neuroglia, in the immune tissues (Campagnoni et al., 1993; Fritz and Kalvakolanu, 1995). Golli-MBP protein molecules (33–35 kDa), unlike classical MBP isoforms, include a 130-amino acid golli domain that is encoded by I-III exons, whose mRNAs are transcribed from upstream promoter (Feng et al., 2000). These isoforms are not normal components of the myelin sheath since they are mainly localized in the nuclei and cell bodies of oligodendrocyte precursors cells (OPC). Mainly the expression of Golli-MBP isoforms is detected during early development and followed by the expression of the classic MBP. The exact function of the Golli isoforms is not fully understood. However, several studies have shown that Golli-MBP mediates the entry of Ca²⁺ ions into oligodendrocytes through voltage-dependent ion channels in a depolarized state, and it is also involved in the differentiation and migration of OPC during myelination (Jacobs et al., 2005). The nuclear localization of Golli-MBP is explained by its binding to transcription factors (Fernandes et al., 2004); however, its role in the regulation of gene expression is still controversial.

Classical MBP protein is represented by six major isoforms in mice and four in humans (Harauz and Boggs, 2013). These proteins are derived from alternative splicing of a single MBP mRNA, consisting of seven downstream exons of the Golli gene complex. The production level of various MBP isoforms is determined by the developmental stage of oligodendrocytes, for instance, isoforms encoded by mRNA with exon-II, which correspond to 17.22, 20.2, and 21.5 kDa in mice, are produced in the onset of myelin formation (Smith et al., 2013). The exon-II negative MBP variants (14 and 18.5 kDa in mice), in contrast, present later in development (Pedraza, Fidler, Staugaitis, and Colman, 1997). Also, exon-II containing MBP isoforms are not involved in the compact myelin formation. At the same time, other exon-II negative variants of MBP (14 and 18.5 kDa in mice) are present in myelin sheath composition (Alinquint, Staugaitis, D’Urso and Colman, 1991). Probably this heterogeneity of MBP isoforms is necessary for developmental regulation of myelogenesis.

Among other things, the diversity of MBP post-translational modifications is another determining factor of its heterogeneity, the role of which is unknown or poorly understood (Kim et al., 2003). Some protein post-translational modifications are reversible while others are not. To date, it is well known that MBP in vivo undergoes five forms of post-translational modification: acetylation, methylation, phosphorylation, citrullination and deamidation (Boggs, 2006). Modification of the MBP protein contributes to the formation of “charge isomers”, which always give many bands on electrophoresis. Normally, human MBP 18.5-kDa occurs as a series of C1–C8 isomers in order of decreasing total positive charge (Zand et al., 1998). The C2 isomer corresponds to deamidated MBP (Kim et al., 2003), while C3–C6 can be modified by combinations of deamidation, phosphory-
lation, and citrullination (deimination); the last two types of modification are expected to have particular functional significance (Boggs, 2006). Covalent attachment of phosphate groups to serine and threonine residues of MBP occurs in response to various extracellular signals, with the greatest changes observed in mature myelin (Zand et al., 1998). Phosphorylation of MBP in the myelin sheath changes in response to neural action potential, and in oligodendrocyte culture in response to extracellular ligands and depolarization (Murray and Steck, 1984; Boggs, 2006). It is assumed that phosphorylation of MBP isoforms containing the sequence encoded by exon-II can inhibit their transport into the nucleus (Pedraza et al., 1997). Reducing the cationic charge of MBP by phosphorylation influences protein conformations as well as protein — lipid and protein — protein interactions. Phosphorylated MBP has a decreased ability to assemble actin in vitro (Boggs et al., 2006), but an enhanced ability to polymerize and bundle tubulin (Harauz and Libich, 2009). It is believed that MBP phosphorylation is not a spontaneous process, since the degree of protein modification changes during the development of the organism, ageing and pathological processes. For instance, the level of MBP phosphorylation in the brain of patients with multiple sclerosis (MS) is significantly reduced (Kim et al., 2003).

Removal of arginyl residues by peptidylarginine deiminase (PAD) leads to the formation of citrulline — an important irreversible modification of MBP (Kim et al., 2003). In the C8 isoform, at least 6 of the 19 Arg residues are citrullinated, which reduces the positive charge of the protein from +19 to +13 — this, in turn, leads to disruption of protein folding, weakening of the contact of MBP molecules with phospholipids, and degradation by proteases such as cathepsin-D (Vassall et al., 2016). The proportion of MBP protein presented in a citrullinated isoform is significantly higher in children than in adults. These data give reasons to believe that they play a regulatory role in the formation of the myelin sheath rather than in its functioning. For example, a healthy brain normally contains 20% of citrullinated forms of MBP, while an increase of their quantity can serve as a marker of the development of multiple sclerosis (Boggs, 2006). In addition, MBP has recently been identified as a target for citrullination in the brains of prion-infected patients with Creutzfeldt-Jakob disease and patients with Alzheimer’s disease (Ishigami et al., 2005; Jang et al., 2010).

Although post-translational modifications of MBP are distributed along the entire length of the protein sequence, their highest frequency is limited to regions that are presumably disordered. Post-translational modification hotspots are mostly represented in the N- and C-terminal regions of MBP, which correspond to exons I and V–VII, respectively (Harauz and Libich, 2009). These modifications, especially phosphorylation and citrullination, contribute to a decrease in the total protein charge. The stability of the multilayer proteolipid myelin sheath is determined by the initial electrostatic interactions of MBP with membrane lipids (Raasakka et al., 2017; Vassall, Bamm and Harauz, 2015). In this regard, it can be assumed that due to a decrease in the forces of intramolecular electrostatic repulsion mediated by modification, the protein undergoes conformational changes, which can affect its ability to bind to the membrane, and these changes, in turn, affect both the formation of myelin and its stabilization. For instance, in the MS brain, deiminated as well as methylated variants of MBP are often found (Kim et al., 2003). For this reason, some researchers classify demyelination as a “post-translational disease”.

Role of MBP in myelination dynamics throughout life

White matter generation begins in late embryonic and the first six postnatal weeks in rodents. Myelin-forming oligodendrocytes arise from multipotent NG2-progenitor cells, also called oligodendrocyte precursor cells (OPCs), which persist throughout life within the adult brain parenchyma and make up around 5% of the glial cell population (Dawson, Levine, and Reynolds, 2000; Levine, Reynolds, and Fawcett, 2001). Since myelin acts as an insulator for the transmission of action potential along the axon, an active process of myelination occurs in the early postembryonic stages of development during active cognitive processes; this is necessary for accelerating information transfer in neural circuits and sustaining axons’ activity (Hasegawa et al., 1992). Myelin sheaths are formed only around the axons of actively functioning neurons, thus in adult cortex, areas remain with unmyelinated or partially myelinated axons. The learning process of a child and an adult, associated with the activation of certain neurons, is accompanied by de novo myelination. (Monje, 2018; Williamson and Lyons, 2018). For instance, human studies provide compelling evidence of a link between adaptive myelination and motor learning, like juggling (Scholz, Klein, Behrens and Johansen-Berg, 2009) or piano practicing (Steele et al., 2013). Elevated motor circuit activity entails not only generation of new oligodendrocytes (McKenzie et al., 2014) but also increased expression of MBP in white matter areas underlying the motor cortex (Sampaio-Baptista et al., 2013). MBP production is proposed to modulate WM plasticity in response to learning-induced neuronal activity by regulating the myelin sheath thickness (Martini and Schachner, 1997). It is worth noting that activity-regulated myelination is not restricted to the motor system, and also includes non-motor learning, as it was revealed in the studies of effects of adult
secondary language learning (Schlegel, Rudelson and Tse, 2012). Thus, de novo myelination is supposed to be an important form of the neuron circuits plasticity.

Myelination, as a developmentally regulated process, in general requires coordinated expression of many genes, including MBP. As mentioned above, MBP is an upstream product of the Golli-MBP gene complex, expression of which is differently regulated both during development and within specific tissues (Campagnoni et al., 1993). Golli-MBP mRNAs are prenatally expressed in oligodendrocytes (Campagnoni et al., 1993) as well as some neuronal populations (Landry et al., 1996, Pribyl et al., 1996). Also, its messenger transcript variants were found in the human fetal spinal cord, thymus, and spleen (Pribyl et al., 1993). For this reason, the function of Golli-MBP isoforms extends beyond the regulation of myelin formation and includes oligodendrocyte proliferation and migration (Jacobs et al., 2005; Paez et al., 2011). Due to this functional ambiguity and spatial distribution in early-developmental stages, Golli-MBP has been called a "molecular link" between the nervous and immune system (Pribyl et al., 1993). Expression of the classic MBP isoforms, in contrast, dominates in later stages of CNS development. In the human prenatal brain development, there were two distinguished periods of MBP expression: one in onset and the other during myelination (Zecović, Andjelković, Matthieu and Tosić, 1998). In the mouse brain the peak accumulation of mRNA encoding MBP occurred in the third postnatal week, thereafter its level declined to a much lower steady state (Zeller et al., 1984). Notably, in the mouse CNS a positive correlation was observed between the MBP production level and CNS myelin sheath thickness, which emphasizes MBP as an essential limiting factor in myelination (Shine et al., 1992). However, the ratios of MBP isoforms vary during development too. The level of expression of exon-II positive MBP messenger RNAs, coding 21.5 and 17 kDa polypeptides, dominates at early postnatal ages, while exon-II negative isoforms, i.e., 18.5 and 14 kDa, are present in the more mature brain (Carson, Nielson and Barbarese et al., 1983). Since exon-II containing MBP molecules are not an integral part of compact myelin, it is believed that these isoforms may play a regulatory role in the process of oligodendrocyte proliferation via a mechanism that relies on their dynamic nuclear import and export (Pedraza, Fidler, Staugaitis and Colman, 1997). Moreover, these mRNAs serve as precursors in biogenesis of myelin-forming exon-II negative MBP isoforms, which are obtained via splicing out exon-II of the primary transcript (Campagnoni et al., 1993). Among other things, an increase of exon-II containing MBP transcripts coincides with the earliest histological signs of remyelination — the process of myelin sheath recovery of previously demyelinated nerve fibers. A 13-fold higher level of expression of exon-II containing MBP transcripts was noted at the beginning of remyelination relative to control white matter mRNAs level. In the later stages of myelin recovery these molecules were replaced by exon-II negative spliced variants (Campagnoni et al., 1993). Thus, the dynamics of the expression of different forms of MBP during remyelination coincide with myelination at the early stages of CNS development. Nevertheless, despite an abundance of MBP isoforms produced through development, it is supposed that the shortest 14 kDa MBP variant might be sufficient for normal development and functioning of myelin. Studies on homozygous shiverer (shi/shi) mice have shown that transgenic production of the small 14 kDa MBP isoform partly recovered individuals from the shiverer phenotype with elimination of tremor and increased lifespan (Kimura et al., 1989).

The content of myelin across the lifespan is not constant. In the human brain, total white matter volume reaches its peak by the age of 30 and then declines with ageing (Miller et al., 2012), correlating with cognitive impairment that mostly affects working executive memory and the slowing of processing speed (Peters, 2002). Under the microscope, age-related degenerative abnormalities in myelin sheaths are revealed as myelin balloons (Feldman and Peters, 1998) and splits of the lamellae at the major dense line (Peters, 2002). Reasons for this are not clear, however, this could be a consequence of a complex of factors, such as reduced OPC differentiation (Sim, Zhao, Penderis and Franklin, 2002), differential vulnerability of oligodendrocytes to accumulation of DNA damage and oxidative stress (Tse and Herrup, 2017), altered lipid metabolism (Lefèvre-Arbogast et al., 2021), and decreased nutrient and energy availability for myelinating cells (Sams, 2021). In addition, senescence-dependent abnormalities in myelin are often caused by a decrease in the expression level of various genes, including MBP, which serves as a robust indicator of brain age. Studies on the human visual cortex of healthy people have shown that classic MBP isoforms gradually increase up to 42 years and then decline during ageing, while Golli-MBP protein production, in contrast, increases (Siu, Balsor, Jones and Murphy, 2015). Significant age-related reduction in MBP levels was revealed in specific layers of the hippocampus (Ahn et al., 2017) — the part of the limbic system critical for learning and memory. In addition, overall age-related decline of MBP was identified in the corpus callosum and in the dorsal column of the spinal cord in aged rats (Xie, Zhang, Fu and Chen, 2013). Taking into consideration the importance of MBP in sustaining myelin, this age-related protein downregulation obstructs further myelin formation de novo and myelin remodeling, which leads to reduction in the conduction velocity of nerve fibers and diminution in neural connectivity.

It is important to note that myelin breakdown through ageing is associated with astocytes and mi-
croaglia activation, which are involved, among other things, in the degradation of myelin components (Xie, Zhang, Fu and Chen, 2013). The inclusions of MBP and proteolipid protein (PLP) were identified within microglial cells of ageing wild-type mice (i.e., up to 24 months old). Also, western blot analysis of purified microglia from 12-month-old mice revealed sarcosyl-insoluble high molecular weight aggregates of MBP (Safaiyan et al., 2016; Thériault and Rivest, 2016). Based on these results, it was assumed that microglia participate in myelin debris uptake that accumulates in the ageing brain. Lysosomal degradation plays a special role within this, because in studies on RAB7 knockout mice with impaired lysosomal activity, MBP-rich puncta in microglia appeared earlier than in wild-type ageing mice (i.e., at 9 months old rather than 18 months old). A significant accumulation of MBP aggregates in ageing microglia indicates impaired clearance capacity. It is still not completely known whether this is a consequence of the senescence of microglia itself or if the accumulation of MBP aggregates is caused by the overloading of myelin debris in the cellular environment, which normal cells cannot cope with utilizing. In some ways this impaired microglial degradative capacity is reminiscent of aberrant accumulation of amyloid proteins in microglial cells during amyloid-induced neurodegenerative disorders, such as Alzheimer's (Paresce, Chung and Maxfield, 1997) or Huntington diseases (Franklin, Clarke and Patani, 2021), which can be explained by the high stability and resistance of amyloid fibrils to enzymatic proteolysis (Kushnirov, Dergalev and Alexandrov, 2020; Schönfelder et al., 2021).

Models of MBP folding within compact myelin

There are various data and ideas about how exactly MBP connects the opposite membranes of myelin and contributes to its compaction. Difficulties in the study of these issues are primarily related to the fact that the formation of compact myelin occurs only in vivo, when the processes of oligodendrocytes are wrapped around axons. All attempts to obtain three-dimensional crystals of 18.5 kDa MBP suitable for X-ray diffractometry have failed, because after removal from tissues, the native form of the protein adopts a random coil conformation and persists as a population of structurally non-identical molecules (Sedzik and Kirschner, 1992). Spectroscopic studies showed that MBP in aqueous solution has a disordered conformation while its secondary structure changes in the presence of lipids or detergents: SDS (sodium dodecyl sulfate) or DPC (dodecylphosphocholine) (Polverini et al., 1999). *In vitro* studies show that different MBP isoforms can interact with a variety of proteins, including cytoskeletal proteins (Bamm et al., 2011; Boggs et al., 2014). At the same time, analysis of the structure of myelin membranes shows that MBP displaces other proteins in the major dense line (Zuchero et al., 2015). The mRNA of membrane-associated MBP isoforms is trafficked to the oligodendrocyte processes plasmalemma, where it is translated and quickly connects to the inner membrane sheets (Bakhti, Aggarwal and Simons, 2014; Seiberlich et al., 2015).

The interaction of MBP with the membrane is mainly based on electrostatic forces between the positively charged amino acid residues of MBP and the negatively charged head groups of the inner leaflet lipids, phosphatidylinerine and phosphatidylinositol 4,5-bisphosphate (Riccio et al., 2000; Nawaz et al., 2009). By binding to the cytosolic membrane surfaces, opposite charges are neutralized, allowing other forces such as hydrogen bonding and hydrophobic factors to be unmasked. Membrane binding switches the properties of MBP, thereby promoting self-interaction into a tightly packed protein phase that forms the major dense line and binds the cytoplasmic surfaces of the bilayers tightly together (Kattwig et al., 2012; Muruganandam et al., 2013). Such a phase transition from a soluble to a polymerized pool of molecules is frequently observed for many structurally disordered proteins, in particular those engaged in RNA binding (Calabretta and Richard, 2015).

An important biochemical feature of the compact myelin composition is a low ratio (0.25) of proteins to lipids, in comparison with the plasma membranes of other cells (ratios ranging from 1.0 to 4.0) (Agarwal et al., 2011). Moreover, studies on the oligodendrocyte cell culture of mutant shiverer mice have shown that the amount of protein in the underdeveloped myelin sheets is significantly higher than in the mature myelin of wild-type individuals (Aggarwal et al., 2011). It is believed that an MBP-formed molecular sieve serves as a diffusion barrier for most cytosolic proteins, including 2’3’-cyclic nucleotide 3’-phosphodiesterase (CNPase) and myelin-associated glycoprotein (MAG) (Aggarwal et al., 2011), and other cellular compartments, to generate oligodendrocyte flat processes. For efficient myelin compaction, it is reasonable to coat the entire cytosolic surface of the oligodendrocyte membrane with MBP molecules, which requires accurate regulation of targeted delivery of large amounts of protein. The membrane-associated translation of the MBP protein in the processes of oligodendrocytes promotes its rapid adhesion to the membrane and conformational stabilization, which is accompanied by flattening of the processes (Simons and Nave, 2016). The MBP assembling within sheaths during myelination, in turn, leads to the disassembly of the actin cytoskeleton (Zuchero et al., 2015). The binding of MBP molecules to membrane phospholipids causes the competitive displacement of gelzolin and cofilin factors, which, being in the cytoplasm after
dissociation with the membrane, triggers depolymerization of the actin cytoskeleton (Zuchero et al., 2015). These intracellular molecular rearrangements ultimately lead to the formation of lipid-saturated isolating myelin sheets containing mainly extracellular PLP and intracellular MBP proteins (Aggarwal et al., 2013).

Transmission electron microscopy studies of MBP/C1 particles adhering to the lipid monolayer combined with three-dimensional reconstruction showed that MBP particles form subtle variations of the basic “C” shape with a diameter of approximately 11 nm in a low salt buffer which mimics the physiological conditions (Beniac et al., 1997). The authors also proposed a model of the molecule topology, which is consistent with earlier three-dimensional models, according to which the MBP comprised five β-sheets in antiparallel configuration and a large proportion of irregular coil (Ridsdale et al., 1997). Later, spectroscopic studies, including multidimensional NMR spectroscopy, of both the full-length 18.5 kDa MBP and their peptides revealed three segments with a strong propensity to form α-helices within a phospholipid environment. Note that all of these models describing the putative structure of MBP are based on the study of the structure of a protein not bound to the membrane. According to in vitro studies based on membrane mimetic model systems, including MD simulations, it has been suggested that the MBP is packed into the major dense line of myelin in a hairpin configuration and a large proportion of irregular coil (Beniac et al., 1997). Later, spectroscopic studies, including multidimensional NMR spectroscopy, of both the full-length 18.5 kDa MBP and their peptides revealed three segments with a strong propensity to form α-helices within a phospholipid environment. Note that all of these models describing the putative structure of MBP are based on the study of the structure of a protein not bound to the membrane. According to in vitro studies based on membrane mimetic model systems, including MD simulations, it has been suggested that the MBP is packed into the major dense line of myelin in a hairpin configuration and a large proportion of irregular coil (Beniac et al., 1997). Later, spectroscopic studies, including multidimensional NMR spectroscopy, of both the full-length 18.5 kDa MBP and their peptides revealed three segments with a strong propensity to form α-helices within a phospholipid environment. Note that all of these models describing the putative structure of MBP are based on the study of the structure of a protein not bound to the membrane. According to in vitro studies based on membrane mimetic model systems, including MD simulations, it has been suggested that the MBP is packed into the major dense line of myelin in a hairpin configuration and a large proportion of irregular coil (Beniac et al., 1997).

Role of MBP in pathological demyelination

The diseases characterized by damage of myelinated covers of nerve fibers in the human CNS are usually grouped into a family of so-called demyelinating diseases. These pathologies can be caused by different factors including inflammatory processes, viral infection, acquired metabolic derangements, ischaemic damage, etc. (Love, 2006). Among demyelinating diseases, the most common is multiple sclerosis (MS) — the autoimmune pathology that commonly affects young and middle-aged adults. MS-mediated myelin degredation leads to disruption of neuron circuits, resulting in a range of neurological and psychiatric symptoms, including cognitive and motor impairment, all of which are determined by the locations of the lesions within the nervous system. The exact etiology of MS is currently unclear, but risk factors include bacterial and viral infections (Marrodan, Alessandro, Farez and Correale, 2019). Apparently, abnormal immune response to an infectious agent causes activation of microglial cells that destroy the blood–brain barrier and myelin sheaths. The destruction of myelin leads to the induction of an autoimmune reaction to a number of proteins, in particular MBP (Lehmann, Rottlender and Kuerten, 2015). MBP-specific antibodies in the blood of patients are one of the main indicators of active demyelination in MS (Cohen, Herndon and McKhann, 1976; Whitaker, 1977). Traumatic brain injuries also provoke myelin destruction, causing the accumulation of MBP in the cerebrospinal fluid and blood, which leads to the development of MS. Myelin damage in MS promotes proteolysis of MBP, resulting in its cleavage into separate fragments, some of which are resistant to further cleavage. Interestingly, the most immunogenic epitope of this protein in MS (Whitaker, 1997) can form amyloid fibrils according to bioinformatic predictions (Aggarwal et al., 2013). It can be assumed that the MBP fragments forming amyloid aggregates are resistant to proteolysis and provoke an autoimmune response during myelin degradation.
It should be noted that MS is accompanied by modifications of the MBP C1, the least modified and most cationic isoform, is the most abundant form of MBP in healthy adult humans; C8, a less cationic isoform with extensive citrullination of arginyl residues, has an increased level in the brain in individuals with MS (Moscarello, Mastronardi and Wood, 2006). Moreover, the severity of MS strongly correlates with the deimination level — 18% against 45% in a normal and chronic MS brain, respectively. Furthermore, in the rapidly progressive and aggressive Marburg variant of MS, the MBP citrullination level reaches 90% (Messe, Boggs and Harauz, 2006). As mentioned earlier, the high positive net charge provides adhesion and stabilization of molecules within phospholipid composition of compact multilayers, which is necessary for myelin retention. When MBP becomes less cationic it is supposed to be unable to carry out this function. In all likelihood, modifications of MBP in MS are not a cause but a consequence of the disease.

### Conclusion

The myelin sheaths are unique structures that ensure the functioning of the most actively working neurons. Modern knowledge about the structure of myelin is far from complete, since this structure is formed only in vivo as a result of the interaction of processes of oligodendrocytes with axons. MBP is a key protein for myelin compaction and function. In this review, we discussed current data and hypotheses about the functional role of MBP, its modifications and conformational changes in the human brain in health and disease. Based on the data obtained in vivo, it can be concluded that this protein binds the opposite membranes of the flattened processes of oligodendrocytes and displaces other proteins from the region of the major dense line. Moreover, binding to membrane lipids provokes aggregation of MBP, which contributes to the compaction of myelin. So far, we can only assume which conformational changes in MBP occur during the formation of compact myelin. In our opinion, the most reasonable and attractive hypothesis is that MBP molecules inside the processes of oligodendrocytes form an amyloid network, which contributes to the reliable isolation of axons associated with myelin. MBP also plays an important role in the development of multiple sclerosis, which is characterized by myelin degradation and induction of an autoimmune response. It is interesting to note that in multiple sclerosis, the most immunogenic epitope of MBP has potentially amyloidogenic properties. Further studies of the structural properties of MBP in vivo will provide progress in understanding the organization and functioning of myelin sheaths in health and disease.

### References


