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Lateral reorganization of myelin lipid domains by myelin basic protein studied at the air–water interface

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Abstract

It has been speculated that adsorption of myelin basic protein (MBP) to the myelin lipid membrane leads to lateral reorganization of the lipid molecules within the myelin membrane. This hypothesis was tested in this study by surface pressure measurement and fluorescent imaging of a monolayer composed of a myelin lipid mixture. The properties of the lipid monolayer before and after addition of MBP into the subphase were monitored. Upon addition of MBP to the monolayer subphase, the surface pressure rose and significant rearrangement of the lipid domains was observed. These results suggest that binding and partial insertion of MBP into the lipid monolayer led to dramatic rearrangement and morphological changes of the lipid domains. A model of adsorption of MBP to the lipid domains and subsequent domain fusion promoted by minimization of electrostatic repulsion between the domains was proposed to account for the experimental observations. The significance of these results in light of the role of MBP in maintaining the myelin structural integrity is discussed.

Keywords: Myelin basic protein; Lipid monolayer; Lipid domains; Protein–lipid interactions; Fluorescence microscopy

1. Introduction

Myelin basic protein (MBP) is an integral component of the myelin sheath in the central nervous system (CNS). It is the second most abundant protein in the CNS, comprising roughly 30% of the proteins found in CNS myelin. MBP is primarily located in the cytoplasmatic spacing of the myelin sheath and is believed to play an active role in stabilizing the periodic myelin structure via non-specific interactions with the apposing lipid bilayers [1–3]. MBP is antigenic and causes a demyelination condition in animal models of multiple sclerosis (MS) [4]. In solution, MBP alone behaves as a random coil without any apparent secondary structures [5]. Although a great deal of effort has been devoted to study the secondary structure of MBP in the presence of surfactants and lipids (see review by Mendz [5]), the results from different studies are not consistent because the MBP folding is strongly dependent on its surrounding environment. It is generally believed, however, that MBP folds into structures containing a higher degree of secondary structures such as α-helices and β-sheets in the presence of amphiphiles compared to its folding in the absence of amphiphiles.

It has been established by numerous experimental studies that MBP interacts strongly with acidic lipids via electrostatic interactions [6]. Under physiological conditions, the 18.5 kDa MBP carries 21 net positive charges. Investigations of the role of MBP in promoting myelin sheath integrity have largely been focusing on its role in promoting inter-membrane adhesion via its interactions with acidic lipid headgroups in apposing membrane surfaces [1,7,8]. Understood to a lesser degree is the role of MBP in creating lateral intra-membrane heterogeneity. It has been suggested that the lipids in the myelin membrane form microscopic domains around MBP [6,9] and that MBP partially inserts into myelin membrane via hydrophobic interactions with the lipid hydrocarbon tails [10]. These two effects may act in concert to promote strong binding of MBP to the myelin membrane: the insertion of MBP into the membrane could induce a localized rearrangement of the lipid molecules, which then form microdomains around the protein. These microdomains formed

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by myelin lipids surrounding the MBP are analogous to membrane rafts, which have been suggested to exist in a wide range of biological systems [11,12].

Several Langmuir monolayer studies of the interactions between MBP and various model myelin lipid mixtures have been reported in literature [13–18]. The kinetics of MBP binding to dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylserine monolayers have been measured [18]. The insertion of MBP into tissue-extracted myelin lipid mixtures revealed surface pressure dependent insertion behavior [13,15,19]. In an attempt to probe the abnormalities in myelin lipids and MBP in control and MS samples, the phase behaviors of MS myelin lipids and normal myelin lipids at the air–water interface were compared [16]. Most recently, the behavior of MBP in modulating the surface viscosity of myelin lipid monolayer was reported [20].

Although it has long been recognized that the interaction of MBP to myelin lipids contain electrostatic and hydrophobic components, the precise location of MBP within the myelin sheath remains to be determined. It is conceivable that non-specific interactions between MBP and myelin lipids occur on the length scale of molecular dimensions and are difficult to probe experimentally. Using a monolayer of myelin lipids at the air–water interface to mimic the actual myelin membrane, we attempted to characterize the role of MBP to create lateral lipid compositional heterogeneity within the myelin lipid membrane. Surface pressure and monolayer domain morphology were measured up to a few hours with and without the addition of MBP to the subphase. A simple theoretical model, which is largely based on electrostatic interactions between the dipolar lipid molecules and the charged MBP, was applied to interpret the experimentally observed domain attraction at early times during the monolayer transition. We believe that our measurement of MBP interaction with the myelin lipid monolayer provides a macroscopic proof to the hypothesis of the lateral MBP/myelin lipid interactions within the myelin membrane. The results from the combined surface pressure and fluorescence microscopy measurements presented in this paper demonstrated that the binding and insertion of MBP to the myelin lipid monolayer and subsequent lateral rearrangement of the myelin lipid domains occurred via non-specific interactions of the MBP with the lipids. To our knowledge, this is the first time direct visualization of the lateral rearrangement of myelin lipid monolayer domains due to MBP binding has been reported.

2. Materials and methods

2.1. Materials

Details of the preparation and sources of the lipid components making up the myelin lipid mixtures used in the study have been given elsewhere [1] and will not be repeated here. Briefly, seven different classes of tissue-extracted lipids were mixed to mimic the experimentally measured lipid compositions in human myelin [21] as shown in Table 1. The fluorescent dye 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (Texas Red-PE®) was purchased from Molecular Probes (Eugene, OR) and was mixed with the myelin lipid mixture at 1% mole fraction. The 18.5 kDa MBP C1 was kindly provided by Dr. Mario Moscarello's laboratory at the University of Toronto, which was purified according to previously published protocol [22]. The lyophilized form of the protein was stored at −20 °C until use. For all measurements, MBP was dissolved in MOPS buffer (150 mM sodium nitrate, 10 mM MOPS sodium salt, pH 7.4). The MOPS buffer was made with Milli-Q water, which was purified by a MilliPore Grandiant A10 system (Bedford, MA) and had resistivity ≥18.2 MΩ cm and total organic content ≤5 ppb. All measurements were carried out using MOPS buffer as the subphase. The chemicals were used as received without further purification.

Table 1
Composition of myelin lipid mixture

	Lipid mole fraction
Phosphatidylcholine (PC)	0.109
Phosphatidylserine (PS)	0.011
Phosphatidylethanolamine (PE)	0.156
Sphingomyelin (SM)	0.079
Cholesterol (Chol)	0.491
Cerebrosides (CE)	0.108
Cerebroside sulfate (CBS)	0.046

The measurements were performed separately on two home-made troughs: one for myelin lipids pressure–area isotherm measurements [23] and one for myelin lipids–MBP interactions [24]. A Nikon Eclipse E800 fluorescence microscope was used for fluorescence microscopy imaging (Nikon Instrument Group, Melville, NY). The fluorescence images from the microscope were fed to a Cohu image intensified CCD camera (San Diego, CA), which was directly connected to the microscope. The black-and-white fluorescence images were recorded using a Panasonic super VHS VCR (Secaucus, NJ). Video images were digitized on a PC installed with ATI 128 PCI 32 MB video card and All-In-Wonder software (Ontario, Canada).

2.2. Probing the MBP–myelin lipid interactions

All experiments involving MBP were done in a miniature trough [24], which was small enough to rest on the translation stage of Nikon fluorescent microscope. Due to instrumentation limitations, no simultaneous measurements of monolayer surface pressure and fluorescence microscopy were conducted. Instead, each experiment was divided into two stages: (1) monitoring of surface pressure during MBP insertion into myelin lipid monolayer and (2) fluorescence microscopy of monolayer morphology. The myelin lipid monolayer was first compressed to the desired surface pressure of ∼25 mN/m and allowed to equilibrate while the monolayer surface pressure was measured by a portable Nima pressure sensor (Coventry, UK). Once the surface pressure was stabilized, ∼100 μL of MBP solution (concentration 0.6 mg/mL) was slowly injected into the subphase. The bulk concentration of MBP in subphase was 0.1 μM. The surface pressure was continuously recorded during protein injection and