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Lateral self-assembly of 18.5-kDa myelin basic protein (MBP) charge component-C1 on membranes

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ABSTRACT

Myelin basic protein (MBP), particularly the classic 18.5-kDa isoform, is a major structural protein of the myelin sheath of the central nervous system. It is an intrinsically disordered, peripheral membrane protein that shows structural polymorphism in combination with several overlapping interaction sites. Here, double electron–electron resonance (DEER) spectroscopy, in combination with a simplified, semi-quantitative analysis based on Monte Carlo simulations, is used to determine the distance distribution of murine 18.5-kDa MBP, unmodified charge component-C1, on large unilamellar vesicles of a lipid composition mimicking the cytoplasmic leaflet of myelin. Three singly spin-labeled MBP variants and a mixture of singly-labeled MBP variants are used. The MBPs, each bearing only one spin label, exhibit average intermolecular distances that are significantly shorter than the distances expected when assuming a random distribution at the employed lipid-to-protein ratios, indicating self-assembly on the membrane. The distribution of elliptical pervaded areas (hard ellipses) on a two-dimensional surface can serve as a model of the nonspecific self-assembly process. The corresponding pair correlation functions $g(r)$ are determined from Monte Carlo simulations with variation of various parameters such as the ellipses' aspect ratios. Comparing the $g(r)$ values with the DEER-derived distance distributions, the pervaded volume is best characterized by a nearly elliptical projection onto the membrane, with an aspect ratio of approximately 1.5, and with the longer semi-axis of approximately 1.4 nm. The approach of using local information from DEER with low-resolution models derived from Monte Carlo simulations can be applied to study the lateral self-assembly properties of other protein complexes on membranes.

1. Introduction

Myelin basic protein (MBP) is a prominent representative of intrinsically disordered proteins, *i.e.*, proteins that lack an ordered and stable tertiary structure, at least in part, and whose inherent flexibility allows them to interact with a variety of binding partners and to be multifunctional, *e.g.*, as hubs in signaling networks [1–7]. Along with the transmembrane proteolipid protein, MBP (primarily the classic 18.5-kDa splice isoform) represents one of the most abundant proteins assembling the myelin sheath, the dielectric insulation surrounding axons of the adult central nervous system of higher vertebrates [8,9]. With a diameter of up to 50 μm, a length of up to 750 μm between nodes, and a lipid content of ~70% by dry weight, the myelin sheath facilitates the rapid transmission of nervous signals. Structural aberrations of this complex, multilamellar arrangement of lipids and proteins can lead to impairment of nerve conduction in the central nervous system, and are involved in neurodegenerative diseases like multiple sclerosis (MS) [10,11].

As a peripheral membrane protein, MBP acts primarily as a "molecular glue" and contributes significantly to the overall stability and integrity of the compacted multilamellar structure of the myelin sheath by interconnecting individual membranes, in addition to many other functions in cytoskeletal assembly and signaling [3,4,12]. The molecular details of how MBP molecules interact with the lipid bilayer and with each other within the sheath are still unknown. In this context, self-assembly of MBP [13–16] and specific, additional interactions with divalent metal ions like Zn^{2+} and Cu^{2+} have been discussed [17–22]. Optical waveguide light-mode spectroscopy has indicated clustering of MBP on artificial myelin membranes [23]. Cell

Abbreviations: CW, continuous-wave; DEER, double electron–electron resonance; DSPC, distearoylphosphatidylcholine; DSPG, distearoylphosphatidylglycerol; EPR, electron paramagnetic resonance; ESE, electron-spin-echo; IDP, intrinsically disordered protein; LPR, lipid-to-protein ratio; LUV, large unilamellar vesicle; MLV, multilamellar vesicle; MBP, myelin basic protein; MS, multiple sclerosis; MTS-SL, [1-oxyl-2,2,5,5-tetramethyl-D-pyrroline-3-methyl]methanethiosulfonate; rmC1, recombinant murine 18.5-kDa MBP charge component C1; X-band, microwave frequency range of ~9.1–9.8 GHz

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biological studies involving immunofluorescence microscopy have suggested the idea of MBP forming a "molecular sieve" on the myelin membrane, implying already the formation of a two-dimensional network of the protein [24,25]. Other intrinsically disordered proteins, such as tau, have also been described to self-assemble on lipid bilayers, a phenomenon due in part to charge screening and increasing the local concentration [26].

Efforts to crystallize MBP in stable and pure three-dimensional crystals for structural determination have been unsuccessful [2,27], and it was realized in the last decade that MBP is intrinsically disordered, conformationally adaptable to diverse binding surfaces and protein partners, and structurally polymorphic even in bound form [4–6]. Less is known about inter-protein interactions within myelin, although it has been investigated for some time [28–30]. Cryo-transmission electron microscopic (TEM) studies of two-dimensional arrays of hexahistidine-tagged MBP on artificial lipid monolayers revealed structures with a diameter of about 3 nm–4 nm, and lamellar-like arrangements featuring characteristic 4.8 nm repeats [2,31–33]. This method features a characteristic resolution of several nanometers, or requires the existence of intermediate to long-range order. Thus, only a rough estimate of the dimensions of MBP (or its quaternary assemblies) could be achieved. Reconstitution of MBP with large unilamellar lipid vesicles (LUVs) under physiological buffer conditions yields an assembly closely mimicking the natural environment of the myelin sheath, where natural lipid–protein electrostatic and hydrophobic interactions are retained. Our solid-state EPR (electron paramagnetic resonance) and NMR spectroscopic studies have provided further information on membrane-interacting amphipathic α-helical segments, as well as on mobile regions (Fig. 1) [4–6,34–39].

With our present knowledge, we have depicted schematic models of adhesion in which the N-terminal and C-terminal segments of 18.5-kDa MBP interact with separate and apposed leaflets of the myelin membrane (see Fig. 8 in reference [38], and Fig. 1 in reference [40]), in a major dense line of roughly 3 nm thickness. This idea is presented in schematic form in Fig. 2A without imposing any detailed tertiary structure of the protein. However, there may be further levels of compaction leading to dynamic molten globule-like structures, partly induced by zinc-binding [20,22,31,32]. Moreover, dimers have long been suggested to be the form which adheres membranes (Fig. 2B). MBP has been cross-linked in the dimeric form in myelin and further analysis indicated that the dimers were anti-parallel [28,29]. We contend, however, that the monomeric form of 18.5-kDa MBP is sufficient to hold together two membrane leaflets (Fig. 2C) [41,42]. We have observed lateral self-assembly of the protein on lipid monolayers [31–33], and hexameric assemblies have been suggested under some *in vitro* conditions [15,16,30]. It has thus been proposed that myelin membrane adhesion is facilitated by MBP–MBP interactions, which can be either lateral (on the same membrane surface) or between two MBP molecules, each adhering to a different membrane leaflet [13,14,24,25,28]. Thus, two major features of 18.5-kDa MBP within myelin remain to be determined: the protein's overall three-dimensional shape, and its degree of self-association. Two potential scenarios of lateral two-dimensional assembly are depicted in Fig. 2D and E.

Electron paramagnetic resonance spectroscopy on spin labels introduced *via* site-directed spin labeling (SDSL) is a powerful method for the characterization of unstructured (or only partly structured) macromolecular systems. This can be achieved by probing the local environment of spin probes, the dipolar couplings between them, and the hyperfine interactions with nuclear spins within their surroundings. Spin labels like the commonly used methanethiosulfonate have been shown to have negligible perturbations even on well-structured proteins [43,44], and would be expected to have minimal effects on the dynamic and "fuzzy" conformation of intrinsically disordered proteins such as MBP [5,6,34,35,45]. The advantage of EPR spectroscopic methods is that they do not require long- or intermediate-range order, and can characterize intra- and intermolecular interactions on a length scale up to ~8 nm, and on the picosecond to microsecond time scale of rotational reorientation [46,47]. With sophisticated methods of pulse EPR spectroscopy, an even more detailed study is possible than by the more common continuous-wave (CW) EPR approaches. Here, we present results from electron spin echo-detected (ESE) EPR and nanoscale distance measurements using double electron–electron resonance (DEER) [48,49]. The combination of the pulse-EPR results (especially the distance information obtained from DEER), with Monte Carlo (MC) simulations of the distribution of physical objects on a two-dimensional surface, is used here to

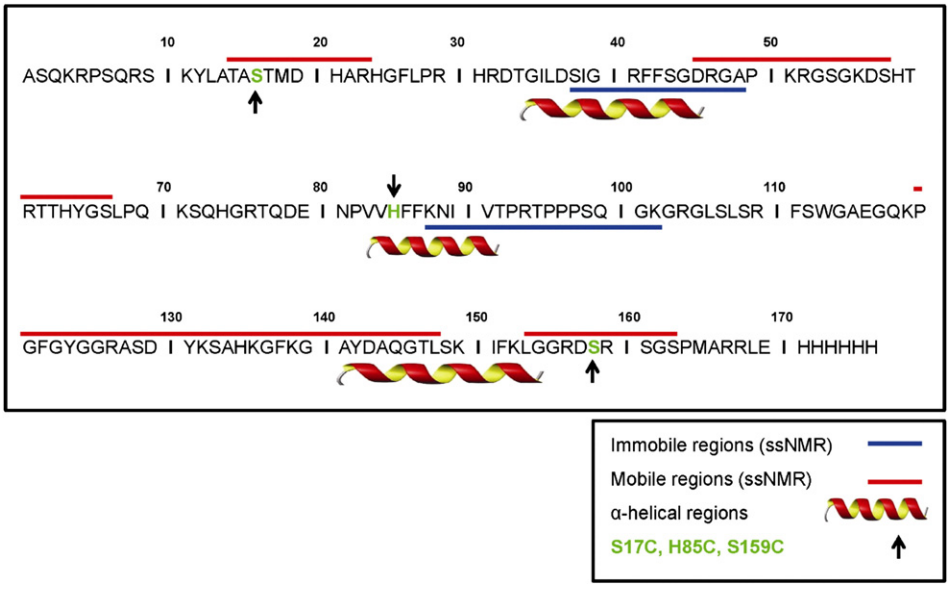

Fig. 1. Amino acid sequence of recombinant murine 18.5-kDa MBP C1-component (denoted rmC1, 176 residues including the C-terminal LEH_6 tag) showing the locations of single Cys-substitutions (S17C, H85C, S159C, indicated by arrows and green font), amphipathic α-helices (indicated by cartoons), regions found by ssNMR spectroscopy to be immobilized in the membrane (blue lines), and regions found by ssNMR to be mobile and exposed to solution (red lines) [6,34,35,37,38,42]. The amphipathic α-helices show strong hydrophobic moments [4] and have been observed by solution NMR spectroscopy to exist in membrane-mimetic conditions [37]; the central and C-terminal helices have been confirmed to interact with myelin-mimetic membranes by site-directed spin labeling and CW-EPR spectroscopy [35,36,38], with further experimental evidence provided by solution and ssNMR spectroscopy [40,67].