Biophysics Interdepartmental Group
PhD. Thesis Defence

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"DYNAMICS AND INDUCED STRUCTURE OF INTRINSICALLY DISORDERED MYELIN PROTEINS BY NMR SPECTROSCOPY"

ABSTRACT

The detailed delineation of structure and dynamics of proteins is a prerequisite for comprehension of their biochemical roles *in vivo*. The 'structure-function' paradigm has had to be refined by the realization that a significant fraction of eukaryotic proteins have substantial regions of disordered structure, yet are involved in critical processes within the cell. The members of myelin basic protein family (MBP), both classic and golli, are identified as being intrinsically disordered, and participate in a diversity of important roles in the central nervous system. This thesis is devoted to the investigation of the structure and dynamics of different members of the MBP family.

Solution nuclear magnetic resonance (NMR) spectroscopy was used to achieve sequence-specific resonance assignments of the recombinant murine BG21 golli isoform of MBP (rmBG21) in physiologically relevant buffer (100 mM KCl). Chemical shift indexing (CSI) revealed only some small fragments having a slight tendency towards α-helicity, which may represent putative recognition motifs. The analysis of backbone dynamics using ^{15}N spin relaxation measurements confirmed the general behaviour of the protein as an extended polypeptide chain. The N-terminal golli-specific portion (residues S5-T69) and the glycine-rich segment A126-G129 were exceptionally flexible. The high degree of flexibility of this N-terminal region may be to provide additional plasticity, or conformational adaptability, in protein-protein interactions, while the A126- G129 region may function as a hinge.

Solid-state NMR (SSNMR) and Fourier transform infra-red (FTIR) spectroscopy were used to delineate structural propensities and dynamics of the classic 18.5 kDa isoform of MBP in association with actin microfilaments and bundles. FTIR spectroscopy showed induced folding of both protein partners. Actin showed some increase in β-sheet content, while MBP showed increases in both α-helix and β-sheet content, with considerable extended structure remaining. SSNMR spectroscopy specifically located these elements in the terminal fragments of MBP, and in a central immunodominant epitope.

SSNMR was utilised to achieve a comparative study of the central immunodominant epitope of the recombinant murine MBP C1 mimic (rmC1) and its deiminated C8 mimic (rmC8) in association with model membranes. This epitope is part of an antigenic peptide that is known of its high affinity to T cells and is thought to trigger the autoimmune phase of multiple sclerosis (MS). Our measurements showed that the epitope adopted an α-helical conformation in both protein variants (albeit with shorter length than was previously reported) and that the helix was less motionally restricted (as a result of its cytoplasmic exposure) in the quasi-deiminated rmC8 compared to rmC1. Our studies as a whole have revealed that MBP is conformationally polymorphic, a structural state that is in consistence with its roles as a linker and signalling hub.

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