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Letter to the Editor: Backbone resonance assignments of the 18.5 kDa isoform of murine myelin basic protein (MBP)

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Biological context

Myelin basic protein (MBP) is a family of developmentally-regulated and translocatable isoforms involved in formation of the myelin sheath of the central nervous system (Campagnoni and Skoff, 2001). The 18.5 kDa isoform of MBP is the most common in adult humans and exists as a series of highly post-translationally modified charge isomers (Kim et al., 2003). The *in vivo* environment of this isoform is the major dense line of myelin, where it maintains the cytoplasmic leaflets of the oligodendrocyte membrane in close apposition. Deiminated MBP is a candidate autoantigen in multiple sclerosis. Other MBP isoforms potentially have roles in signalling pathways during myelin development, yet our understanding of these phenomena is limited by the lack of detailed structural knowledge.

All MBP isoforms are 'intrinsically unstructured' to facilitate their interactions with diverse ligands (Hill et al., 2002). The protein is primarily a flexible coil in aqueous solution, but attains ordered secondary structure in the presence of detergents and lipids, as well as in organic solvents such as trifluorethanol (TFE) (Liebes et al., 1975). Recently, site-directed spin labelling and electron paramagnetic resonance experiments on a recombinant form of the 18.5 kDa isoform of murine MBP (rmMBP, 176 residues, M_r 19421.5 Da) in protein-vesicle pellets have defined how different regions of the protein interact with myelin-like membranes (Bates et al., 2004). We have turned to solution NMR in order to define its tertiary structure, and present here the first NMR assignment of the backbone of uniformly $^{13}C^{15}N$-labelled rmMBP.

Methods and experiments

The 18.5 kDa rmMBP (unmodified qC1 form, 176 residues including a C-terminal LEH_6 tag) was expressed in *E. coli* BL21-CodonPlus(DE3)-RP cells (Stratagene, La Jolla, CA), and purified as previously described with some modifications to improve yield (Bates et al., 2002). Upon growth in M9 minimal media supplemented with $^{15}NH_4Cl$ and $^{13}C_6$-glucose (Cambridge Isotope Laboratories – C.I.L., Cambridge, MA), a yield of 10 mg of purified, uniformly $^{13}C^{15}N$-labelled rmMBP was obtained per litre of culture. The protein was dissolved in 30% TFE-d_2 (C.I.L.) in water at a concentration of 2 mM, and NMR studies were undertaken at 25 °C on a Bruker Avance spectrometer operating at a proton frequency of 600 MHz. Two-dimensional ^1H-^{15}N HSQC data (Figure 1), and a combination of triple-resonance spectra using several complementary pulse sequences (HNCO, CBCANH, CBCA(CO)NH, HCC(CO)NH) were obtained (Sattler et al., 1999). The ^1H and ^{13}C chemical shifts were referenced to TSP (3-(trimethylsilyl)-propionic acid-d_4 sodium salt) in a capillary tube, and ^{15}N chemical shifts were referenced indirectly to liquid ammonia. Backbone assignment was performed using AUREMOL (www.auremol.de) (Gronwald and Kalbitzer, 2004) and PACES (Coggins and Zhou, 2003) software.

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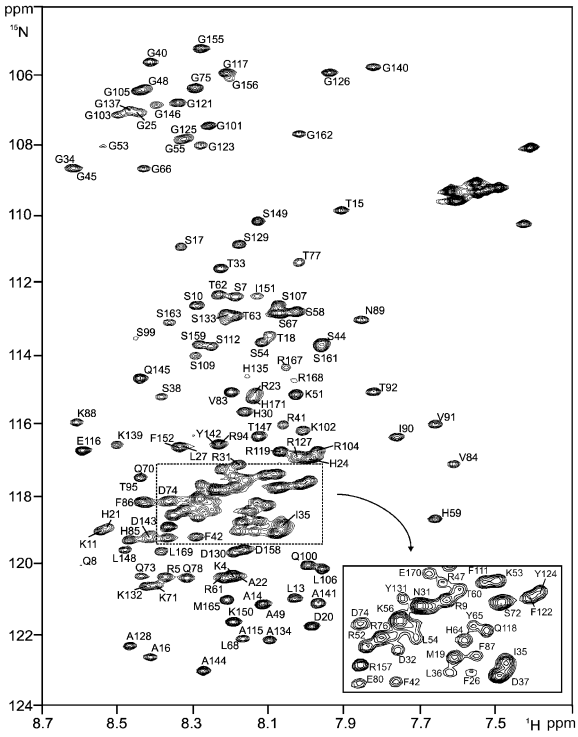

Figure 1. The ^{1}H-^{15}N HSQC spectrum of uniformly ^{13}C^{15}N-labelled rmMBP (176 residues, including a C-terminal LEH_6 tag) dissolved in 30% TFE-d_2 in water at a concentration of 2 mM. A total of 151 of 164 expected backbone peaks are assigned (there are 11 prolyl residues).

Extent of assignments and data deposition

Initial NMR experiments were performed with the protein dissolved in either 50 mM sodium phosphate buffer, pH 7.5, 250 mM NaCl in 90% H_2O/10% D_2O, or in 100% TFE-d_2, or in 30% TFE-d_2 in water. Under the latter conditions, the protein exhibited excellent solubility and stability, and superior dispersion and resolution of the proton and nitrogen resonances in the two-dimensional ^{1}H-^{15}N HSQC spectrum (Figure 1). Assignment of 151 backbone peaks has been completed (of a possible 164 backbone peaks since there are 11 prolines), including the first histidyl residue of the hexahistidine tag (Figure 1). The segment comprising murine residues Val83-Thr92 that represents an immunodominant epitope that forms an amphipathic α-helix positioned on the surface of the lipid bilayer (Bates et al., 2004). Chemical shift index analysis using Cα, Cβ, and CO data confirmed that this region was also α-helical in 30% TFE-d_2. Following this helix is a proline-rich region from Pro93-Pro98, comprising a MAP kinase site, in which residue assignment was ambiguous due to incomplete residue data. The region bounded by residues Leu106 to Gly114 was especially difficult to assign due to incomplete connectivity data.

The challenge in NMR studies of MBP is to define preparation conditions that appropriately mimic its natural environment and allow it to attain a defined fold, yet which make the experiments tractable. This isoform of MBP is already nearly 20 kDa in size, has a tendency to precipitate, aggregates lipid vesicles, and forms multimeric complexes with detergents. Thus, initial NMR studies of MBP must be done using solution approaches and organic solvents, with 30% TFE-d_2 being the best condition so far defined since it yields stable, reproducible, high resolution data. In a CD spectroscopic study, a set of conformational transitions of MBP was observed at increasing concentrations of TFE, the first occurring at roughly 30% TFE, and the second at roughly 85% TFE (Liebes et al., 1975). TFE is frequently used in NMR experiments of both membrane-associated and globular proteins to stabilise α-helices, and the relatively low concentration used here represents a reasonable membrane-mimetic environment that makes it possible to perform a first direct structural determination of MBP *via* solution NMR.

The ^{1}H, ^{13}C, and ^{15}N chemical shifts for backbone resonances have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) with accession number 6100.

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