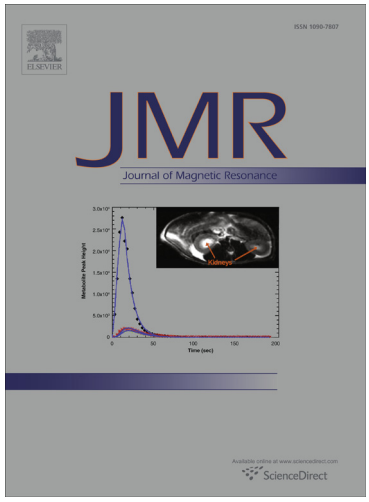

A bifunctional spin label reports the structural topology of phospholamban in magnetically-aligned bicelles

Jesse E. McCaffrey, Zachary M. James, Bengt Svensson, Benjamin P. Binder, David D. Thomas

PII: S1090-7807(15)00301-8
DOI: http://dx.doi.org/10.1016/j.jmr.2015.12.005
Reference: YJMRE 5766

To appear in: *Journal of Magnetic Resonance*

Received Date: 17 September 2015
Revised Date: 25 November 2015

Please cite this article as: J.E. McCaffrey, Z.M. James, B. Svensson, B.P. Binder, D.D. Thomas, A bifunctional spin label reports the structural topology of phospholamban in magnetically-aligned bicelles, *Journal of Magnetic Resonance* (2015), doi: http://dx.doi.org/10.1016/j.jmr.2015.12.005

A bifunctional spin label reports the structural topology of phospholamban in magnetically-aligned bicelles

Jesse E. McCaffrey, Zachary M. James, Bengt Svensson, Benjamin P. Binder, David D. Thomas

Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN 55455, USA
Keywords: bifunctional spin label, phospholamban, bicelles, orientation, EPR, molecular dynamics

ABSTRACT: We have applied a bifunctional spin label and EPR spectroscopy to determine membrane protein structural topology in magnetically-aligned bicelles, using monomeric phospholamban (PLB) as a model system. Bicelles are a powerful tool for studying membrane proteins by NMR and EPR spectroscopies, where magnetic alignment yields topological constraints by resolving the anisotropic spectral properties of nuclear and electron spins. However, EPR bicelle studies are often hindered by the rotational mobility of monofunctional Cys-linked spin labels, which obscures their orientation relative to the protein backbone. The rigid and stereospecific TOAC label provides high orientational sensitivity but must be introduced via solid-phase peptide synthesis, precluding its use in large proteins. Here we show that a bifunctional methanethiosulfonate spin label attaches rigidly and stereospecifically to Cys residues at i and i + 4 positions along PLB's transmembrane helix, thus providing orientational resolution similar to that of TOAC, while being applicable to larger membrane proteins for which synthesis is impractical. Computational modeling and comparison with NMR data shows that these EPR experiments provide accurate information about helix tilt relative to the membrane normal, thus establishing a robust method for determining structural topology in large membrane proteins with a substantial advantage in sensitivity over NMR.

1. Introduction

Magnetically-aligned bicelles are a powerful tool for studying the structural topology of integral membrane proteins by magnetic resonance spectroscopy. Reconstitution into aligned bicelles can resolve the anisotropic spectral properties of nuclei for NMR or nitroxide spin labels for EPR, yielding valuable information regarding protein orientation with respect to the bilayer. Bicelles (bilayered micelles) are mixtures of long- and short-chain phospholipids that combine to form flat patches of long-chain lipid bilayers bordered by the short-chain lipids [3]. Due to the negative magnetic susceptibility ($\Delta\chi$) of bicelles, at high magnetic field ($B_0 \geq 1$ T), they align spontaneously with the membrane normal (**n**) perpendicular to the field ($\mathbf{n} \perp \mathbf{B_0}$). Alignment is enhanced by the addition of lanthanides that associate with the bicelle surface and further decrease $\Delta\chi$ (Dy^{3+}), or increase $\Delta\chi$ (Tm^{3+}) to a positive value, flipping the bicelles such that they align with **n** parallel to the field ($\mathbf{n} \parallel \mathbf{B_0}$) [3].

In solid-state NMR spectroscopy, particularly $^{1}H/^{15}N$ PISEMA and SAMMY experiments, membrane protein alignment in bicelles (or mechanically-oriented bilayers) resolves $^{15}N - ^{1}H$ dipolar coupling and ^{15}N chemical shift anisotropies to permit the determination of transmembrane helical tilt and azimuthal rotation [4]. Similarly, membrane protein alignment in EPR can resolve the *g*-factor and hyperfine coupling anisotropies of nitroxides introduced via site-directed spin labeling, and optimization of long-chain lipid composition can produce excellent bicelle alignment in EPR, despite much lower magnetic field values than in NMR [5]. EPR offers a substantial advantage over NMR in sensitivity and can be applied to much larger proteins. However, conventional Cys-linked spin labels typically exhibit intrinsic sub-microsecond rotational mobility relative to the peptide backbone, resulting in motional averaging that limits their utility in EPR for determining peptide backbone orientation and rotational dynamics.

To provide accurate information about protein backbone rotational dynamics [6] and orientation of membrane proteins, we have used the spin label TOAC (2,2,6,6-tetramethylpiperdine-1-oxyl-4-amino-4-carboxylic acid), which suppresses backbone-independent probe motion by incorporating the nitroxide group and αcarbon into the same six-membered ring [7]. Using TOAC, the nanosecond rotational rates and amplitudes of membrane protein domains have

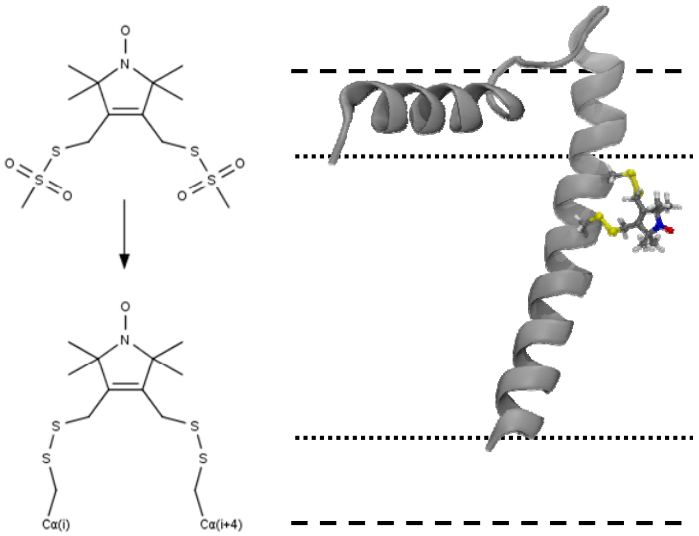

Figure 1. Left: structural formula of BSL before and after reaction. Right: energy-minimized structure of BSL attached to Cys at positions 32 and 36 on monomeric PLB, based on the NMR structure [1]. BSL dihedral angles were initialized from an x-ray crystal structure of BSL attached to a helix on T4 lysozyme [2], then further refined by molecular dynamics simulations to produce the shown structure. The bilayer normal is indicated by **n**, the bilayer surface by dashed lines, and dotted lines indicate the approximate boundaries of the hydrophobic interior.