Membrane and Ligand Binding of the Amino-proximal Domain of Prion Protein Defined by EPR

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Introduction

Prion protein (PrP) is best known as a factor that is essential for host susceptibility to transmissible spongiform encephalopathies (TSEs) or "mad cow" diseases [1]. In TSEs, PrP undergoes a major structural reorganization from its normal monomeric form to an oligomeric and sometimes fibrillar state. The intrinsically disordered amino-proximal domain of PrP contains four copies of a highly conserved octapeptide sequence, PHGGGWGQ (Fig 1A). This octarepeat region mediates the binding of sulfated glycans, which influence the conversion of PrP to pathological forms, and hemin – a toxic byproduct produced from the trauma that occurs during a stroke (Fig 1B). Electron Paramagnetic Resonance (EPR) is being employed to investigate the conformational transitions of the octarepeat region, and its interaction with hemin and lipids.

Experimental

EPR data were collected at the NHMFL on a Bruker E680 EPR spectrometer.

Results and Discussion

Here we describe the EPR studies of PrP peptides OP63C (PHGCGWGQPHGGGWGQ) and OP71C (PHGGGWGQPHGCGWGQ) in lipids, and their behavior in buffers of different pH levels and their interactions with hemin. EPR spectra revealed that the peptides bind to hemin at pH 5, and this hemin binding immobilizes the peptides (Fig 1B). To be noted, W-band EPR spectra at 95 GHz are more sensitive to fast motion than X-band at 9 GHz, which is very suitable for this study. EPR spectral line shape in the presence of POPC/POPG (4:1) liposomes revealed that the peptides bind to the lipids (Fig 1C) at pH 5 and pH 5.5. Surprisingly, there is no interaction with the lipid for both OP63C and OP71C peptides at neutral pH. This is of interest since decreased pH is associated with tissue damage and cell death during a stroke. EPR power saturation measurements were also performed to determine the immersion depths and membrane orientation of the peptides. It was observed that OP63C sits on the lipid head group, and OP71C is immersed in the lipid acyl chains (not shown).

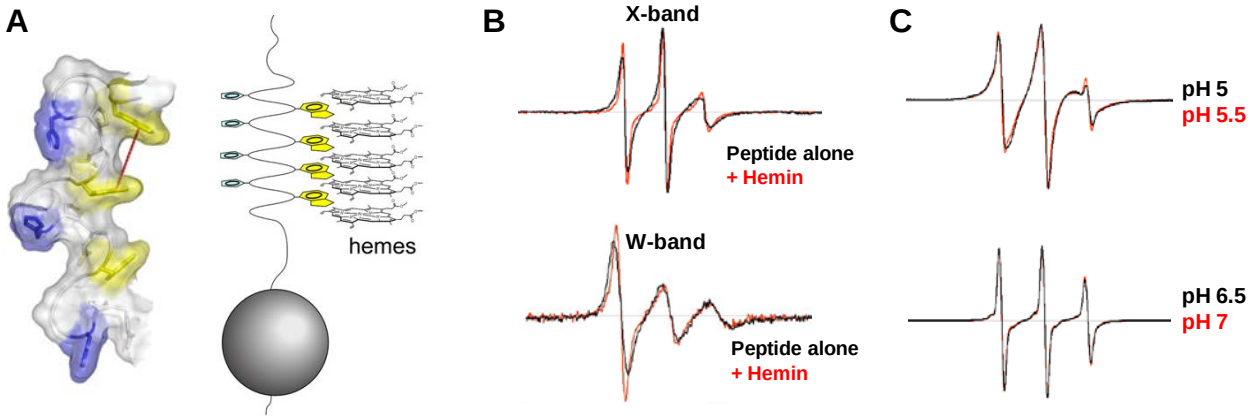

Figure 1. (A) Solid surface representation of the octarepeat region of prion protein (left), and the proposed interactions of PrP with planar aromatic molecules (right). (B) X- and W- band EPR spectra of OP63C. The X-band EPR spectra were measured from samples in 40% glycerol. (C) EPR spectra of OP71C peptides in lipids at different pH values.

Conclusions

EPR spectroscopy at two different microwave frequencies was used to characterize the octarepeat region of prion protein and its interaction with lipids and hemin. These results have important implications in efforts to design novel therapeutic peptides to alleviate hemin toxicity following cardiovascular trauma, such as stroke.

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References

[1] Taubner, L.M., *et al.*, J. Mol. Biol., **395**, 475-490 (2010).