Interaction of the Amino-Proximal Domain of Prion Protein with Lipids and Ligands Defined by EPR

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Introduction

An example of a disease-associated protein with an intrinsically disordered domain is prion protein (PrP) [1]. PrP has an ability to adopt two distinct conformations. The first PrP form is a pathogen that causes fatal neurodegeration. In contrast, the second form, referred to as cellular or non-pathological prion protein, has been implicated in a number of critically important cellular functions, including neuroprotection and recovery from cardiovascular injury. The 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, copper, and hemin. The latter is a toxic byproduct generated during traumatic injury events, including stroke. Electron paramagnetic resonance (EPR) was employed to investigate the mobility and conformational transitions of the octarepeat region and its interaction with hemin and lipids.

Experimental

OP71C (PHGGGWGQ-PHGCGWGQ), a peptide that contains the octarepeat region of prion protein with a single cysteine substitution at residue 71, was synthesized and labeled with EPR spin probes. EPR data were collected at the NHMFL on a Bruker E680 spectrometer.

Results and Discussion

First, EPR spectra were used to determine lipid and ligand binding of the OP71C peptides. The comparison between EPR spectral line shape in the presence and absence of POPC/POPG (4:1) liposomes revealed that the OP71C peptides bind to the lipids (Fig. 1B) at pH 5, and their lipid partition is affected by peptide to lipid ratio (data not shown). EPR spectra also show that the peptides bind to hemin at pH 6.3, and this hemin binding immobilizes the peptides. As shown in Fig. 1C, broadened EPR spectra were observed in the presence of hemin. Hemin titration experiments also suggested that each peptide binds to 5 to 6 hemin molecules (Fig. 1D). In addition, EPR power saturation measurements were performed to determine the membrane immersion depths of the peptides. OP71C was found buried in the lipid acyl chains (data not shown).

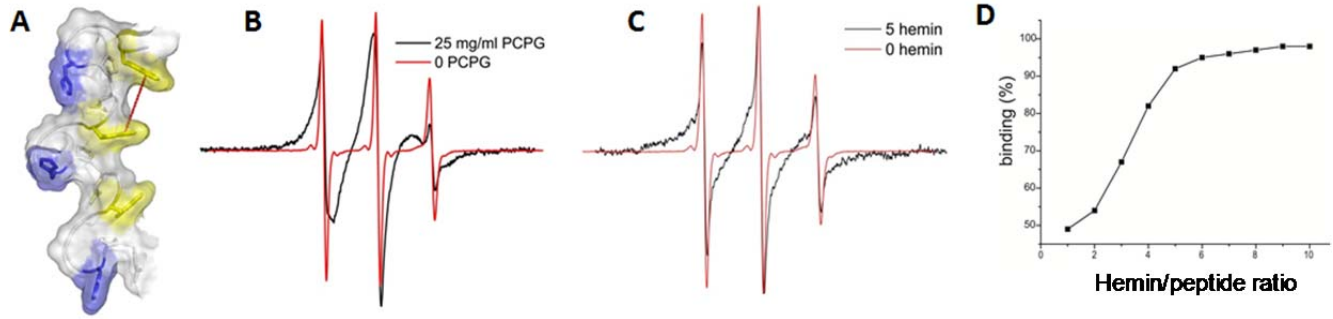

Figure 1. (A) Solid surface representation of the octarepeat region of PrP, with histidines shown in blue, and tryptophans in yellow. (B) EPR spectra of OP71C peptides binding to POPC/POPG lipids (black), and in solution without lipids (red) at pH 5. (C) EPR spectra of OP71C peptides alone (red) and with 5-fold Hemin (black). (D) Hemin binding to OP71C at pH 6.3 detected by EPR.

Conclusions

Using EPR, we characterized the octarepeat region of prion protein and its interactions 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).