Interaction of the Amino-Proximal Domain of Prion Protein with Hemin 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 or "mad cow" diseases [1]. It has an ability to adopt two distinct conformations. The first form is a pathogen that causes fatal neurodegeration. By contrast, the second one, 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. 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.

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

To characterize the interaction between hemin and the prion octapeptide, we collected the EPR spectra of the OP71C peptides with and without hemin. The EPR spectra of the OP71C peptides with spin-labeled R1 side chains show three sharp peaks, which is consistent with peptides tumbling freely in solution (Fig. 1A). By contrast, the spectra of OP71C in the presence of excess hemin is broadened, which suggests slower motion of OP71C and R1 upon hemin binding. To determine hemin-peptide binding affinity, OP71C peptides were titrated with up to 10 fold molar concentration of hemin (Fig. 1B). The hemin titration data were fitted by DynaFit. The best fitting shows three hemin binding sites on the OP71C peptide, with dissociation constants $K_1 = 8.6 \times 10^{-1}$ M, $K_2 = 1.3 \times 10^{-5}$ M and $K_3 = 1.0 \times 10^{-7}$ M, respectively. In addition, hemin-peptide interaction was also investigated by EPR power saturation measurements. Fig. 1C shows a typical power saturation curve of the OP71C peptides (black square). Notably, this saturation effect is largely relieved after adding hemin (grey circle), which acts as a relaxing agent. This indicates that hemin is located in close proximity to the R1 side chain of the OP71C peptide.

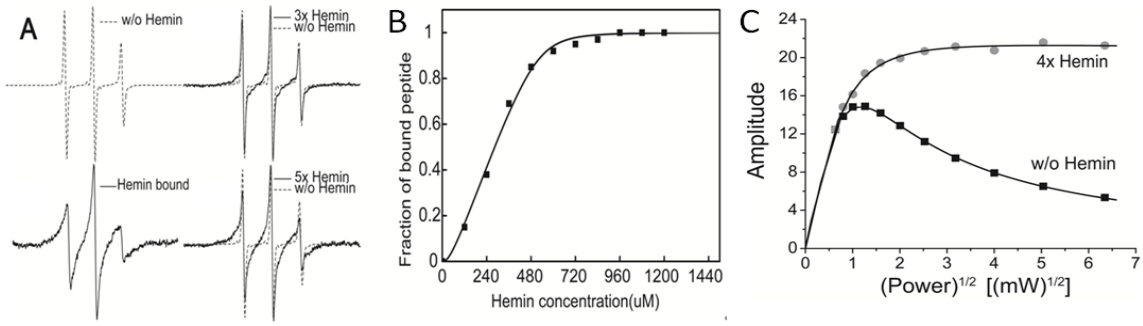

Fig. 1. (A) EPR spectrum of spin-labeled OP71C peptides without hemin (dotted line) was compared with the spectra of hemin-bound OP71C (solid line). (B) Titration curve of hemin to 120uM OP71C peptides. Solid curve denotes the best fit. (C) Hemin binding to prion peptides detected by EPR power saturation.

Conclusions

Taken together, the EPR results confirmed hemin-peptide interaction and suggest that each OP71C peptide binds three hemin molecules with positive cooperativity. 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).