Pulsed EPR Analysis of an Ectodomain Region of HIV Envelope Protein gp41 by DEER – Double Electron Electron Resonance

L. Yu, L. Song (NHMFL), E.L. Reinherz (Harvard Med. School)

Introduction

A vaccine capable of stimulating protective anti-viral antibody responses is needed to curtail the global Acquired Immunodeficiency Syndrome (AIDS) epidemic caused by HIV. The membrane-proximal ectodomain region (MPER) of the HIV envelope protein gp41 is the target of four anti-HIV antibodies (2F5, 4E10, Z13 and 10E8). To gain insight into MPER viral function and immunogenicity, detailed structural analysis of the MPER on a lipid environment is of great importance. Our previous NMR and EPR analysis revealed an L-shaped helix-hinge-helix MPER structure. Here, we performed EPR distance measurements to investigate the relative configuration between the N- and C-helix of the MPER peptides.

Experimental

EPR spectroscopy was performed on a Bruker E680 spectrometer. Spin-spin distances were determined by two EPR techniques: conventional EPR (sensitive distance range 8-25 Å) and pulsed EPR (sensitive distance range 15-80 Å). Conventional EPR spectra were collected at 150K while pulsed EPR data were collected at 65 K.

Results and Discussion

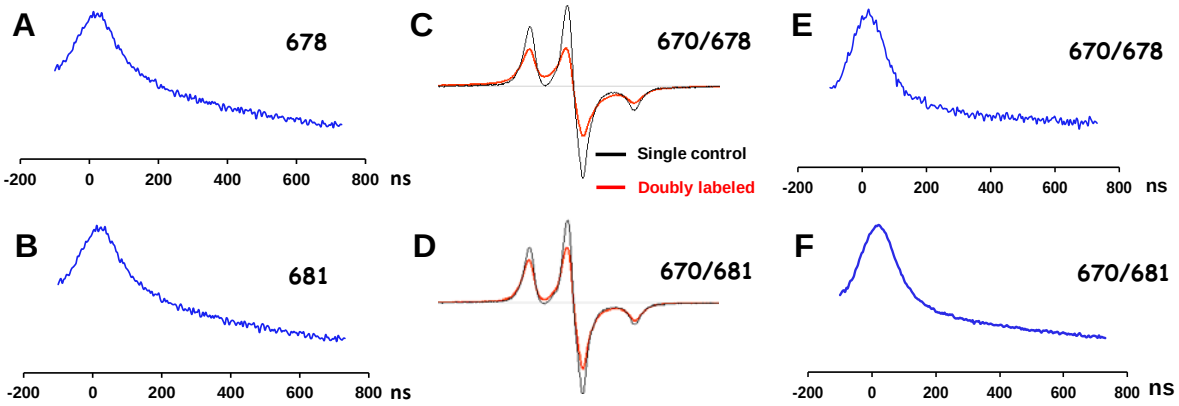

Figure 1. EPR analysis of HxB2 MPER 671A/674A peptides. (A) and (B): pulsed EPR spectra of singly spin-labeled peptides at 678 and 681. (C) and (D): conventional EPR spectra. Spin-spin distance is determined by comparing doubly labeled with singly labeled peptides. (E) and (F): pulsed EPR spectra of doubly labeled peptides.

The EPR distance studies were focused on a wild type HxB2 strain MPER and a non-nature occurring 671A/674A sequence. To characterize HxB2 MPER peptides, two double residue pairs were introduced: 670/678 and 670/681. Residue 670 is located at the N-helix; residues 678 and 681 are located at C-helix. The spin-spin distances for the two residue pairs are consistent with an L-shaped two-helix MPER. Next, MPER peptides with 671A/674A were studied using similar EPR protocols. Surprisingly, short spin-spin distances (< 30 Å) were observed for singly spin-labeled MPER peptides at residue 670, 678, and 681 (**Figure 1**). The results suggested that the peptides formed oligomers. Also, the spin-spin distances for the two residue pairs are significantly different from the values observed from HxB2 MPER peptides. The above results suggest that 671A/674A mutation disrupts the helix-hinge-helix structure of the MPER.

Acknowledgements

This work was supported by NIH grants RO1AI84785 / U19AI91693 (to E.L.R.), a grant from the Gates Foundation (to E.L.R.), and an UCGP grant 5080 (to L.S.).