Investigation of a Specialized Hinge Segment in HIV Envelope Protein Gp41 Using EPR

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Introduction

HIV-1 uses its trimeric gp160 envelope protein consisting of non-covalently associated gp120 and gp41 subunits to mediate entry into human T lymphocytes. A facile virus fusion mechanism compensates for the sparse Env copy number observed on viral particles and includes a 22 amino acid segment at the gp41 base, termed the membrane proximal external region (MPER). Our previous results suggested that the MPER consists of a structurally conserved pair of viral lipid-immersed helices separated by a hinge with tandem joints that can be locked by capping residues between helices. Here, we performed EPR immersion depth and distance measurements to further investigate the configuration of the MPER segment in native HIV variants and a non-natural AA mutant.

Results and Discussion

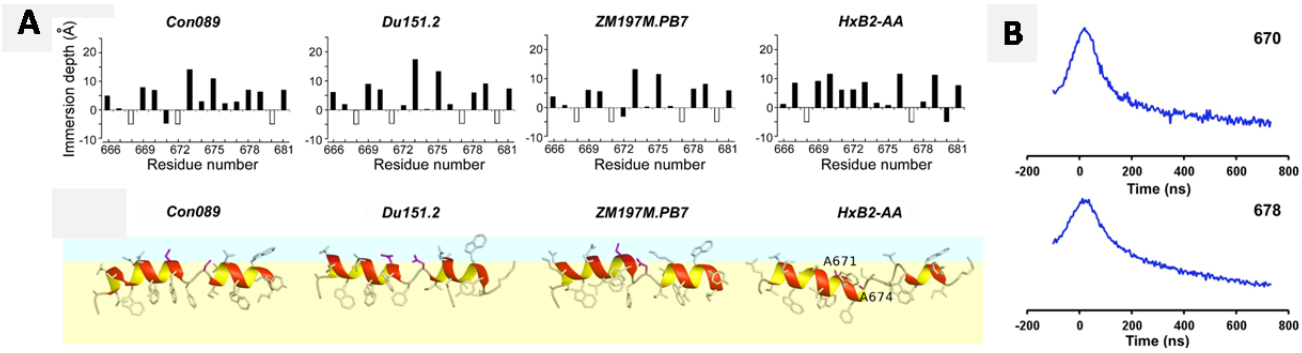

Figure 1. EPR analyses of MPER peptides. (A). Upper panel: EPR membrane immersion depths of the MPER peptides; Lower panel: Structural models of the peptides on the membrane surface (B): EPR DEER spectra of singly spin-labeled peptides at 670 and 678 of AA mutant.

First, we compared the secondary structure and membrane immersion depths of native MPER peptides with different sequences: Con089, Du151.2 and ZM197M (Figure 1A). The results indicate that the MPER peptides are conserved with an L-shaped helix-hinge-helix structure that may be similar among MPERs with different a.a. sequences. To further determine the effect of hinge residue sequence variation on MPER structure, we selected a non-native MPER sequence, 671A/674A. Immunological assays suggested that 671A/674A mutation inhibit cell-cell fusion and viral infectivity (not shown). Here, EPR immersion depth and distance results show that this non-nature occurring MPER sequence adopts a distinct conformation and residual membrane immersion depths (Fig. 1A, upper panel). Figure 1A (lower panel) shows the MPERs embedded in the membrane based on both NMR and EPR results. The HxB2-AA mutant peptide has a deeply buried middle section, bending in a different orientation with respect to other native MPERs. To be noted, short spin-spin distances (< 30 Å) were observed for singly spin-labeled AA mutant MPER peptides at residue 670, 678, and 681 (Figure 1B), suggesting that the peptides formed oligomers. These results have been published online in J. Mol. Biol [1].

Conclusions

Helix-hinge-helix structure is conserved in native HIV MPER segments, but is disrupted by hinge residue mutations. A flexible MPER hinge segment is critical for HIV fusion, and a key feature to design an effective vaccine to elicit protective antibodies.

Acknowledgements

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References

[1] Sun, Z.Y., *et al.*, J. Mol. Biol., S0022-2836(13)00614-1 [Epub ahead of print] (2013).