EPR Analysis of a Membrane-Bound HIV Gp41 MPER Segment as a Vaccine Candidate

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Introduction

A vaccine capable of stimulating anti-viral antibody responses is needed to curtail the global Acquired Immunodeficiency Syndrome (AIDS) epidemic caused by HIV-1. A conserved membrane-proximal ectodomain region (MPER) of the HIV-1 envelope protein gp41 is a target for vaccine design to elicit protective anti-HIV antibodies (Fig. 1A). To date, structural characterization of epitope-paratope pairs has contributed to the understanding of antigenicity. By contrast, few structural studies relate to immunogenicity, the process of antigen-induced immune responses *in vivo*. Using a lipid-arrayed MPER of HIV-1 gp41 as a model antigen, the influence of physicochemical properties on immunogenicity was investigated in relation to structural modifications of MPER/liposome vaccines characterized by EPR spectroscopy.

Results and Discussion

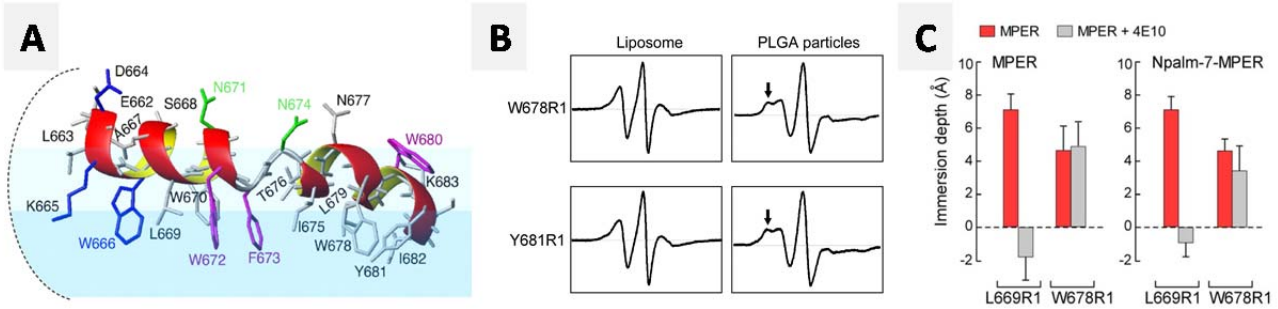

Figure 1. EPR analyses of MPER segments in liposome vaccines. (A). MPER structure on the membrane. (B). EPR spectra of MPER peptides bound to liposomes vs PLGA nanomparticles. Arrows in the latter highlight immobilization. (C). Comparison of membrane immersion depth values of MPER and Npalm-7-MPER peptides in the presence and absence of 4E10 antibody.

First, we have used site-directed spin-labeling and EPR spectroscopy to monitor conformational changes in the MPER arrayed on the surface of liposomes or lipid-coated poly(lactide-*co*-glycolide) (PLGA) nanoparticles. Immobilized components of the spectra were evident in the PLGA particles preparation compared to that of the liposomes (Fig. 1B). This reduced mobility highlights an altered structural property of the MPER peptides when it is arrayed on the lipid-coated nanoparticles. Since an immobile feature is not characteristic of the MPER on virus particles, liposomes were chosen as the vaccine delivery vehicle. Next, EPR analysis was performed to assess changes in MPER orientation that were induced by a 7-residue linker and lipid modification in Npalm-MPER, which will stabilize the peptides in the lipids. The membrane immersion depths of spin-labeled L669R1 and W678R1 in Npalm-MPER were comparable to those of L669R1 and W678R1 in the free MPER segment adsorbed on the surface of the membrane (Fig. 1C). Consistent with 4E10-induced depth change of L669R1 previously observed, L669R1 was lifted out of membrane and exposed to the aqueous phase upon 4E10 binding to Npalm-MPER, suggesting that MPER orientation and antibody interaction is preserved on the surface of Npalm-7-MPER (7 spacer)/liposome. However, sequence modifications affected MPER membrane orientation and antigenic residue accessibility, thereby altering antibody responses (not shown).

Conclusions

The data provide fundamental insights for immunogen design using structural properties, and have important implications for HIV vaccine development. These results have been published in J. Bio. Chem. [1]

Acknowledgements

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References

[1] Kim, M., *et al.*, J. Bio. Chem., **288**, 31888-31901, (2013).