HIV Membrane Analysis by Multi-frequency and High-field EPR at 9, 95 and 240 GHz

L. Yu, H. van Tol, L. Song (NHMFL)

Introduction

Since the acquired immunodeficiency syndrome (AIDS) was recognized in 1981, an estimated 65 million infections and 25 million deaths have been ascribed to human immunodeficiency virus-1 (HIV-1) (CDC, 2006). Preventive vaccination and effective treatment are paramount to eliminate further global HIV-1 spread. The outer membrane of HIV-1 plays a critical role in virus-host cell fusion, which is a major target for vaccine design and therapeutic intervention. Here, multi-frequency EPR at 9, 95 and 240 GHz has been used to define the physical properties of HIV virion mimic membranes, including lipid fluidity and polarity. The improved g-tensor resolution with increasing magnetic field allows enhanced sensitivity to extract side-chain mobility, polarity and membrane order. Since the HIV membranes consist of a mixture of different phospholipids and cholesterol, the effect of cholesterol on HIV membrane properties was also characterized.

Experimental

X-band (9 GHz) and W-band (95 GHz) EPR spectroscopy were performed on a Bruker E680 spectrometer. 240 GHz EPR spectroscopy was carried out using a home-built 12 Tesla spectrometer, all at the EMR Facility. HIV-1 virion mimic membranes were prepared using DOPC/Sphingomyelin/DOPE/DOPS/Cholesterol with the molar ratio of 9:18:19:9:45. Spin-labeled lipids at specific positions (5-Doxyl PC, 7-Doxyl, 12-Doxyl and 16-Doxyl PC) were used to determine membrane fluidity and polarity (**Figure 1**).

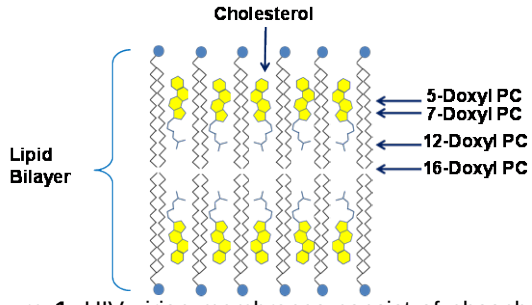

Figure 1: HIV virion membranes consist of phospholipid bilayers and 45% of cholesterol. Spin-labeled lipids at different positions on the lipid acyl chains are indicated.

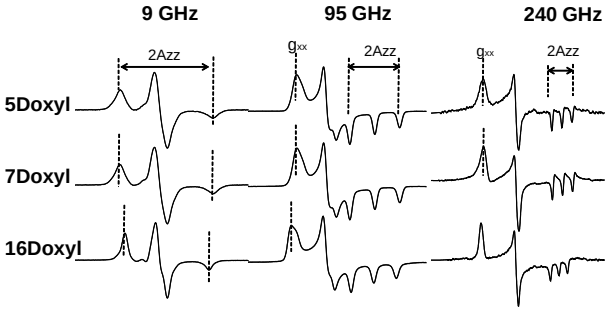

Figure 2: Lipid polarity of HIV virion membranes characterized by multi-frequency EPR at 9, 95 and 240 GHz. Spectra were recorded at 200K.

Results and Discussion

Altogether, the results show that the HIV membrane cholesterol-binding-domain (CBD) is near 5-Doxyl and 7-Doxyl, and distant from 12-Doxyl and 16-Doxyl (not shown). Decreased mobility, enhanced polarity and profound lateral-ordering were identified for the lipids near the cholesterol-binding-domain (CBD) (**Figure 2 and 3**). Cholesterol decreases lipid mobility and increases polarity of the CBD. EPR spectra at 95 and 240 GHz are sensitive to anisotropic motion and lipid lateral ordering (**Figure 3**). In addition, multi-frequency EPR provides additional experimental dimensions to facilitate determination of physical properties.

Figure 3: Lipid fluidity of HIV virion membranes characterized by EPR at 9, 95 and 240 GHz. EPR spectra were recorded at 288 K.

Conclusions

Using site-specifically spin-labeled lipids, the fluidity and polarity profiles across the lipid bilayers of the HIV-1 membranes were obtained, and the cholesterol binding domains (CBD) were characterized. And, multi-frequency EPR is a powerful approach to extract complex structural parameters.

Acknowledgements

This work was supported by an UCGP grant 5080 (to L.S.).