Localization of the HIV-1 Accessory Protein Vpu and Its Submolecular Fragments in Langmuir Monolayers: X-ray Reflectivity and Grazing-Incidence X-ray Diffraction

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Introduction

Vpu is an 81 amino acid integral membrane protein encoded by the HIV-1 genome with a single hydrophobic transmembrane helix and a cytoplasmic domain containing two amphipathic helices within a helix-loop-helix. It enhances the release of virus from the infected cell and triggers degradation of the virus receptor CD4. Vpu's submolecular fragment Vpu_{28-81} contains only the helix-loop-helix of the cytoplasmic domain. Since Vpu is synthesized from bicistronic mRNA and cotranslationally inserted into membrane of the endoplasmic reticulum, it is most desirable to determine Vpu's structure within a membrane-like environment. Langmuir monolayers of phospholipid at the water/helium interface incorporating Vpu protein provide one such model system. Our previous studies of full length Vpu within phospholipid monolayers at the water/helium interface as a function of protein/lipid ratio and surface pressure via x-ray reflectivity (performed on beamline X-22B at the National Synchrotron Light Source at Brookhaven National Laboratory, Upton, NY) provided the electron density profiles for the monolayers.[1] The profiles indicated that the hydrophobic transmembrane domain is oriented approximately normal to the plane of the monolayer within the phospholipid hydrocarbon chain layer thereby progressively disrupting the in-plane ordering of the chains with increasing protein content, as determined by grazing-incidence x-ray diffraction (GID). The profiles also indicated that the helices of the cytoplasmic domain lie with their long axis parallel to the monolayer plane, either on the surface of the phospholipid headgroups in the water subphase or within the headgroup layer depending on surface pressure. A comparative study of Vpu with its submolecular fragments, one of which being Vpu_{28-51}, is an effective way to further test our interpretation of the monolayer electron density profiles in terms of Vpu's structure within the host phospholipid monolayer. In the case of Vpu_{28-51}, it can provide an indication as to whether the interaction of the cytoplasmic domain with the phospholipid monolayer is affected by its short-loop connection with the transmembrane domain within the monolayer since the Vpu_{28-51} possesses only the cytoplasmic domain. In addition, GID will be able to assess the effect of the interaction of the cytoplasmic domain with the phospholipid monolayer in the absence of that of the transmembrane domain.

Results and Conclusions

Initial specular x-ray reflectivity and GID measurements were performed at the Complex Materials Consortium Collaborative Access Team (CMC CAT) employing the unfocused undulator beamline 9-ID-B. The electron density profiles calculated from the reflectivity data for the pure phospholipid 1,2-diligno-ceroyl-sn-glycero-3-phosphocholine (DLgPC) at various surface pressures indicated an increasing thickness and broader chain tilt-angle distribution for the monolayers with increasing surface pressure. The GID from the hexagonal in-plane chain packing was collected in much shorter times with improved statistics, as expected (e.g., peak count rate of 2500 cps vs 50 cps, signal/background 18/1 vs 3/1 between APS and NSLS). The FWHM of the GID peak increased with increasing surface pressure consistent with the increasing width of tilt-angle distribution suggested by the electron density profiles. However, during x-ray exposure, the FWHM of the GID peak broadened and the position of the peak shifted to lower q_{xy}, suggesting a progressive disordering of the in-plane packing of the DLgPC chains with increasing the x ray exposure. Thus, a translation stage under the Langmuir trough was employed to translate the monolayer perpendicular to the incident beam during data collection thereby minimizing the effects of radiation damage in the GID data so collected, such effects being much more pronounced in the GID, as opposed to the reflectivity, data.

Reference

[1] S. Zheng, J. Strzalka, C. Ma, S. J. Opella, B. M. Ocko and J. K. Blasie, Biophys. J. **80**,1837-1850 (2001).

Acknowledgments

We thank the staff of CMC CAT for their assistance. The design and implementation of the LSS at CMC CAT by Scott Coburn, Elaine DiMasi and Ben Ocko (Physics Dept, Brookhaven NL) made this work possible. It was supported by the NIH under grant GM56538. Work at the CMC Beamlines is supported in part by the Office of Basic Energy Sciences of the U.S. Dept. of Energy and by the National Science Foundation Division of Materials Research. Use of the Advanced Photon Source is supported by the Office of Basic Energy Sciences of the U.S. Department of Energy under Contract No. W-31-109-Eng-38.