Localization of the Cytoplasmic and Transmembrane Domains of the HIV-1 Accessory Protein Vpu within Phospholipid Monolayers by X-ray Reflectivity: Comparison of Full-length Vpu_{2-81} with the Submolecular Fragments Vpu_{2-37} and Vpu_{2-51}

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Introduction

Vpu is an 81 amino acid integral membrane protein encoded by the HIV-1 genome with an N-terminal hydrophobic transmembrane domain and a C-terminal hydrophilic cytoplasmic domain. The transmembrane domain enhances the release of the virus from the infected cell. The cytoplasmic domain triggers degradation of the virus receptor CD4. Our previous x-ray reflectivity (XRR) and grazing incidence x-ray diffraction (GIXD) studies of Vpu_{2-81} within phospholipid monolayers indicated that the hydrophobic transmembrane domain is oriented approximately normal to the monolayer plane within the phospholipid hydrocarbon chain layer. The studies also indicated that the amphipathic helices of the cytoplasmic domain lie on the surface of the phospholipid headgroups in the water subphase. We determined to investigate the possible effects of the cytoplasmic domain's interaction with the surface of the host phospholipid monolayer on the transmembrane domain in determining the tertiary structure of the peptide within the monolayer. To do so, we performed a comparative structural study of Vpu_{2-81} with its submolecular fragments Vpu_{2-37} and Vpu_{2-51}, which were truncated to different extents in the cytoplasmic domain, within 1,2-dilignoceroyl-sn-glycero-3-phosphocholine (DLgPC) phospholipid monolayers at the water/helium interface via XRR and GIXD. Although Vpu_{2-81}'s submolecular fragments Vpu_{2-37} and Vpu_{2-51} have been truncated into shorter sequences (namely, 36 residues for Vpu_{2-37} and 51 residues for Vpu_{2-51}), all three proteins possess identical transmembrane domains. In phospholipid micellar and multilayer environments, the cytoplasmic domain of Vpu_{2-81} contains an amphipathic helix-loop-amphipathic helix secondary structure, while Vpu_{2-37} contains only a very short portion of the first amphipathic helix, and Vpu_{2-51} contains the entire first amphipathic helix of Vpu's cytoplasmic domain, as determined by nuclear magnetic resonance (NMR).

Methods and Materials

The amino acid sequence of the recombinant Vpu_{2-81} polypeptide used in this work was QPIQIAIVAL VVAIIIAIVV WSIVIIEYRK ILRQRKIDRL IDRLIERAED SGNESEGEIS ALVELGVELG HHAPWDVDDL. The sequence of Vpu_{2-37} was QPIQIAIVAL VVAIIIAIVV WSIVIIEYRK ILRQRK, and the sequence of Vpu_{2-51} was QPIQIAIVAL VVAIIIAIVV WSIVIIEYRK ILRQRKIDRL IDRLIERAED.

XRR and Qz-resolved GIXD were obtained with the liquid surface spectrometers on beamline X22-B at the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory and at sector 9 at the APS at Argonne National Laboratory. Nearly identical custom Langmuir troughs were used and supported with vibration isolation on the spectrometer's sample axis.

The normalized reflectivity data were fitted by the conventional "slab-model" refinement procedure. The validity of the monolayer electron density profiles obtained via this analysis was checked by using a totally independent "box" refinement procedure.

Results

Electron density profiles derived from the normalized XRR of protein/DLgPC monolayers as a function of the protein/lipid ratio at a constant surface pressure of 45 mN/m have been quantitatively analyzed by using a model calculation for the hydrocarbon chain region and a model fitting for the headgroup region. The quantitative results show that the electron density and thickness of the hydrocarbon chain region of the mixed monolayer increase simultaneously with an increasing protein/DLgPC mole ratio over the entire range of mole ratios for Vpu_{2-51}/DLgPC and Vpu_{2-81}/DLgPC. In contrast, Vpu_{2-37}/DLgPC shows a similar behavior only at the lowest protein/DLgPC mole ratio of 1:50. In fact, the behavior of the Vpu_{2-37}/DLgPC monolayer is qualitatively different, showing a steady increase in electron density of the hydrocarbon chain region but an initial increase followed by a substantial decrease in the thickness of that region over the full range of the protein/lipid mole ratio.

The model fitting for the headgroup region shows that although the widths of the distribution of the cytoplasmic domain's helices within the polar headgroup region of the monolayer profile are relatively constant (~4 Å), the

separation between the centers of the distributions of the cytoplasmic domain helices and the lipid polar headgroups depends strongly on the length of the proteins.

Discussion

On the basis of this quantitative analysis, the localization of the transmembrane domain and cytoplasmic domains within the host lipid monolayer is generally similar for all three proteins. However, the variation in the average tilt angle of the hydrocarbon chains and the transmembrane domain with respect to the monolayer surface normal for $Vpu_{2\text{-}37}$ within the hydrocarbon chain region of the monolayer profile structure with increasing protein/DLgPC mole ratio is substantially different from that for $Vpu_{2\text{-}51}$ and $Vpu_{2\text{-}81}$. In addition, the localization of the helices in the respective cytoplasmic domains of the three proteins with respect to the phospholipid headgroups within the headgroup region of the monolayer profile structure depends strongly on the lengths of their cytoplasmic domains. Thus, the interaction of the cytoplasmic domain of Vpu on the surface appears to determine the tilt of the transmembrane helix within the hydrocarbon chain region of the host phospholipid monolayer.

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Reference

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