Interplay of Cholesterol and Env Protein in the Lytic Deformation of HIV-1 by Peptide Triazoles

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Abstract

Background: HIV-1 Env spike consists of gp120 involved in binding receptors and gp41 which is embedded in a lipid envelope that is mainly cholesterol (45-mol %) and involved in fusion. Envelope cholesterol affects infectivity of the virus and interacts with gp41. Our lab has developed peptide triazoles that inhibit gp120 binding to CD4 and CCR5/CXCR4 and cause leakage of capsid p24, from the viral lumen. Examining the effects of cholesterol on p24 release allows us to investigate lipid-protein interplay at the Env spike.

Methodology: Cholesterol was extracted from BaL.01 (HIV-1) and Vesicular Stomatitis Virus (VSV-G) spike psuedotyped viruses containing the Luciferase gene using methyl β-cyclodextrin (MβCD). Infectivity was detected by chemiluminescence from Luciferase expression in cells while p24 release and gp41 were measured by spinning virus and testing supernatant and pellet fractions respectively by ELISA. Fluidity was measured with Laurdan. Shed gp120 was detected from western blots. Morphological analysis was done on fixed virus by transmission electron microscopy (TEM). Cholesterol was quantified using Cholesterol Oxidase and the fluorescent Amplex Red dye.

Results: Initial depletion of viral membrane cholesterol strikingly enhanced both KR-13-induced p24 capsid protein leakage and infectivity and decreased virus size (TEM). Further depletion of cholesterol arrested both processes and caused complete shedding of gp120 but gp41 content and the virus size remained unchanged and membrane fluidity increased though this wasn't specific to HIV-1. Under similar conditions, the non-virolytic parent peptide HNG-156 did not induce p24 release and the VSV pseudotyped viruses did not see any enhanced infectivity.

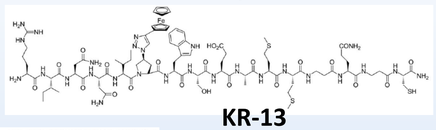
Conclusions: Light cholesterol depletion enhances both KR-13 triggered p24 release and infectivity while heavy depletion arrests both processes, possibly due to shedding of gp120. While fluidity increases during cholesterol depletion, it is not specific to the HIV-1 spike. Correlated trends between infectivity and leakage during cholesterol depletion leading up to the gp120 shedding suggest lipid-protein interplay. To determine the mechanism of enhancement, we will test protease-treated viruses and Env mutations that disrupt the cholesterol interacting (CRAC), transmembrane, and cytoplasmic tail domains interacting with the lipid envelope. Together, this work will help determine the impact of lipid-protein interplay and define approaches to inactivate the virus.

Rationale/Hypothesis

Rationale:

- KR-13 inhibits HIV-1 infection and causes leakage of capsid p24 from the lumen of the virus.
- p24 release requires perturbation of the viral envelope.





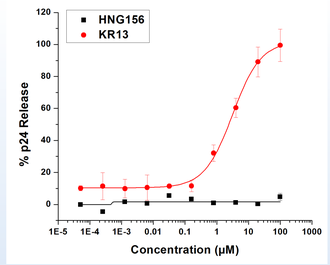
KR-13

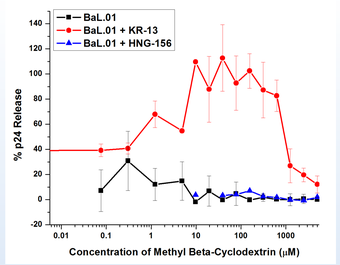


Hypothesis: Since viral envelope is enriched in cholesterol (45 mol-%), its content will affect the extent of HIV-1 lytic inactivation induced by peptide triazole KR-13. To test this hypothesis, cholesterol (45-mol%) was depleted with MβCD.

Bastian 2011, 2013, Brugger 2006, Liu 2008

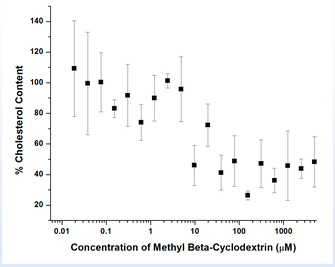
Pseudoviral pre-treatment with MβCD causes bimodal effects on KR-13-triggered p24 release

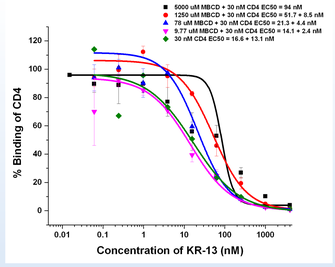




- MβCD pre-treated HIV (BaL.01) displays a bimodal effect (enhancement at low [MβCD] and arrest at high [MβCD]) in p24 leakage by KR-13. [KR-13] is EC_{50} from leakage dose response (left). Data from ELISA.
- MβCD without peptide or non-virolytic peptide HNG-156 does not leak.

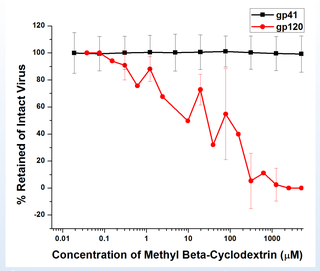
MβCD treatment depletes cholesterol and does not affect KR-13 competition for gp120





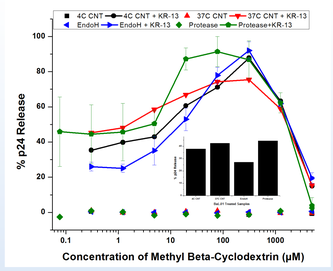
- MβCD treatment of HIV-1 results in depletion of cholesterol with an EC_{50} ~10 μM and a maximum depletion of ~60% using Amplex Red (left).
- MβCD does not impair KR-13 competing with CD4 for gp120 using ELISA.

MβCD treatment results in shedding of gp120 but not gp41



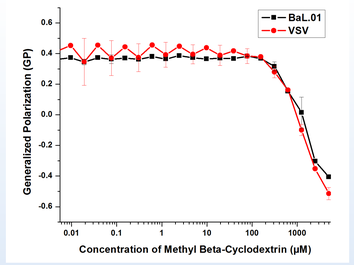
MβCD treatment results in a dose-dependent shedding of gp120 but not gp41 using western blot and ELISA respectively.

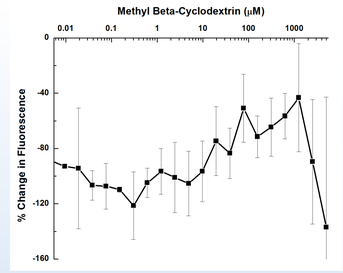
Protease cleaning of spikes does not affect bimodal KR-13-triggered p24 release



Protease removal reduces spike gp120 content but does not affect bimodal p24 release.

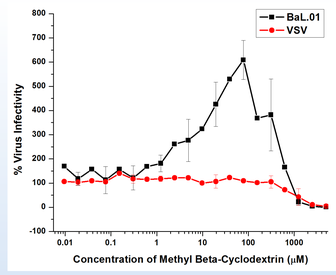
MβCD treatment changes envelope properties





- MβCD treatment results in a more fluid envelope at high [MβCD] using Laurdan (left).
- It also results in fluorescent dequenching of the FRET donor which may suggest the separation of probes located in raft and non-raft phases.

MβCD treatment causes bimodal effects on infectivity



MβCD treated HIV pseudoviruses display two-phase effect on infectivity (enhancement followed by arrest) specific to HIV and not VSV-G.

Conclusions and Future Plans

- Low and high [MβCD] treatment effects are observed; these could be due to different populations of cholesterol being depleted.
- Low [MβCD] effects are specific to HIV-1 Env (enhancement of p24 release and infectivity) while high [MβCD] effects are not (abrogation of infectivity, increase in fluidity).
- Similar trends in KR-13 mediated p24 leakage and infectivity with MβCD treatment suggest similar effects on envelope membrane could underlie both processes.
- Low [MβCD] effects on HIV-1 Env gp120 content indicate that the spike is sensitive to membrane cholesterol content.
- Future work will focus on mutating Env hot spots suggested to interact with the membrane (CRAC, C-terminal tail) to determine mechanism of spike protein sensitivity to the membrane.

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- Bastian, A. R., et al. 2013 Irreversible Breakdown and Inactivation of HIV-1 by Targeting Env gp120 with Peptide Triazoles. *Retvir*
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