Irreversible inactivation and breakdown of HIV1 with Env-gp120 targeting peptide triazole thiols

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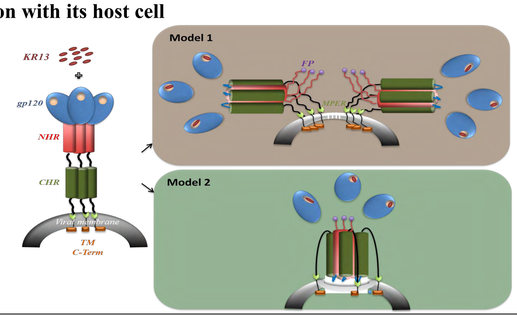
Abstract

Background: Initial entry of HIV-1 into host cells remains a compelling and yet elusive target for developing agents to prevent infection. This step is mediated by a sequence of interactions of a trimeric gp120/gp41 virus envelope (Env) protein complex with host cells. A peptide triazole class of entry inhibitors have shown to bind to gp120 with close to nanomolar affinity, to suppress protein ligand interactions of gp120 with CD4 and co-receptor binding sites, and to inhibit cell infection by a broad range of virus subtypes. We recently found that sulfhydryl-containing peptide triazoles (PT-SH), irreversibly disrupts cell-free virions, leading to virus core release. We seek to understand the mechanism of this cell-free virolysis. Our hypothesis is that the PT-SH hijacks the viral fusion mechanism prior to cell engagement and leads to transient pore formation resulting in irreversible virolysis.

Methodology: The peptide triazoles (PTs) were synthesized using solid phase Fmoc chemistry with click conjugation. The PTs included KR13 (PT-SH); KR13b (KR13 with the thiol blocked); KR13s (containing a scrambled KR13 sequence), and the parent PT, HNG156, with no free thiol. HIV-1 entry inhibition by PTs were compared using a single-round infection assay using lab synthesized pseudoviruses. Release of the Env protein gp120 and nucleocapsid protein p24 from the PT treated virions were tested using western blot and ELISA respectively. The fixed residual virion was tested for gp41 recognition by direct ELISA using antibodies 2F5 and 4E10 and morphological analysis using transmission electron microscopy. To compare the relationship of KR13 induced cell-free virolysis and virus-cell entry, we examined the effect of T20 on the inhibition of KR13 induced virolysis.

Rationale

- In this work we are investigating the mechanism of action of peptide triazole thiol induced cell-free HIV-1 virolysis
- We seek to understand whether there is a relationship between peptide triazole induced HIV-1 virolysis and CD4 induced HIV-1 fusion with its host cell

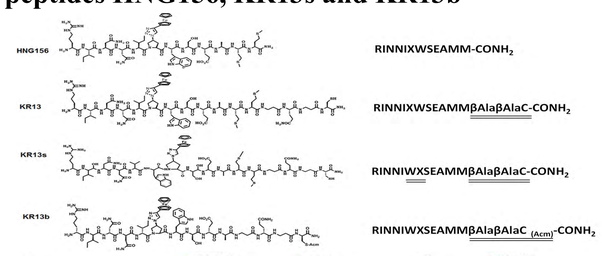


Experimental Objectives

- Compare the anti-viral effects of KR13 vs. control peptide triazoles such as HNG156 (parent peptide) , KR13s (scrambled) and KR13b (Cys-blocked)
- Study the time-dependent inactivation of HIV-1 pseudotyped virion by KR13
- Study the nature of the KR13 treated particles by analyzing the gp41 retained on the particles and the morphology of the residual virion
- Identifying the relationship of KR13 induced virolysis virus-cell entry by testing the sensitivity of other known entry inhibitors (T20) to virolysis
- Understand whether mutations that are sensitive to virus-cell entry have an effect on cell-free virolysis
- Relate the time dependent inhibition of KR13 induced p24 release by fusion inhibitors to the timeline of HIV-1 fusion with a host cell

Results

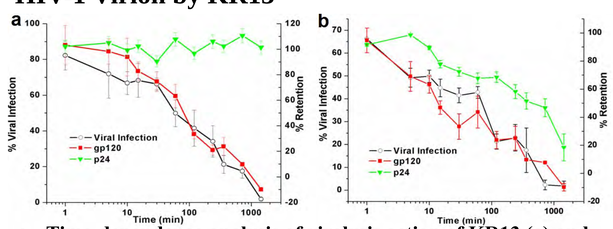
I. Comparing anti-viral effects of KR13 and control peptides HNG156, KR13s and KR13b



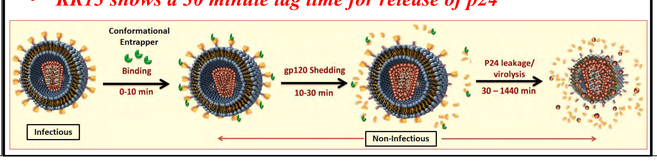
- The figure above shows the structures of virolytic peptide triazole KR13 and the control peptides HNG156, KR13b and KR13
- KR13, HNG156 and KR13b have good viral inhibition EC50s of 55 nM, 102 nM and 830 nM respectively
- All the three peptides KR13, HNG156, KR13b cause similar gp120 shedding , *but only KR13 leads to p24 leakage from the viral core*
- *The table below shows the breadth of the p24 release by KR13 on clade A, B, C and founder HIV-1*

Virus	P24 Release IC50 (μM)
92UG037.8 (A)	0.67 ± 0.24
QF495.23M.Env.A3 (A)	3.34 ± 1.14
TRO-11 (B)	1 ± 0.35
HXBC2 (B)	1.89 ± 2.0
BAL-01 (B)	0.8 ± 0.03
ZM135 M. PL10a (C)	1.86 ± 0.86
CAP 210 (C)	25.6 ± 2.31
ZM246F.C1G (founder)	8.8 ± 1.66
ZM247F fs (founder)	16 ± 2.2
ZM249M.B10.D4 W (founder)	15 ± 1.8

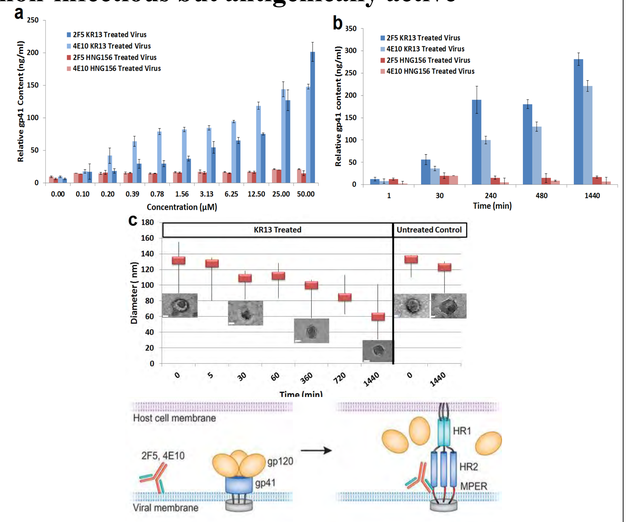
II. Time-dependent Inactivation and Virolysis of HIV-1 Virion by KR13



- Time-dependence analysis of virolysis action of KR13 (a) and HNG156 (b) on cell-free HIV-1 BaL pseudotyped.
- The viral infection inhibition analysis (left y-axis) and viral gp120 and p24 retention is shown on the right y-axis.
- Both KR13 and HNG156 lead to equal rate of viral infection inhibition and gp120 shedding
- *KR13 shows a 30 minute lag time for release of p24*

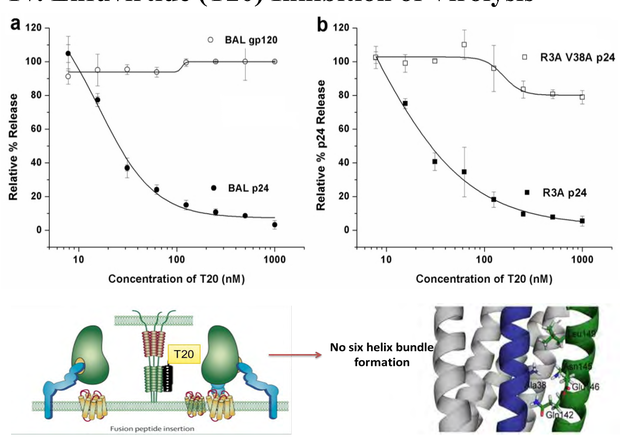


III. Thiol Peptide triazole treated HIV-1 virus is non-infectious but antigenically active



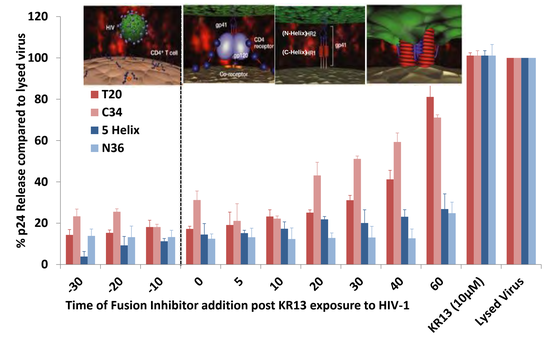
- A. Concentration-dependentceand B. Time dependence analysis of KR13 and HNG156 (parent peptide) treated HIV-1 virion binding to gp41 antibodies 2F5 and 4E10 which react with membrane-associated MPER epitopes at the base of the gp41 ectodomain
- C. Morphological analysis of the time dependent effect of KR13 on HIV-1 virus using transmission electron microscopy (n=16).
- Both show more than *80% gp41 retention* on the residual virion even after 24 hours of treatment. The KR13 treated virions were smaller, with a *>50% reduction* in average diameter compared to untreated virions.

IV. Enfuvirtide (T20) Inhibition of Virolysis



- *T20 competes out KR13 induced HIV-1 BaL A. and HIV-1 R3A B. wild type virolysis at an IC50 of 15 nM*, using KR13 at constant concentration of 1μM
- The V38A mutant is a T20 inhibition resistant mutant
- Since both sCD4 and KR13 have been shown to lead to gp120 shedding, this enhances the exposure of the T20 binding site on the viral spike

V. Time Dependent Addition of Fusion Inhibitor



- The fusion inhibitors T20, C34 , 5 Helix and N36 were added to gradient purified HIV-1 either prior to (-30, -20, -10) or post exposure of KR13
- The steps of fusion are shown as an insert in the graph in order to relate fusion to KR13 induced p23 release
- *The data shows that fusion inhibitors that target the C-Helix inhibits KR13 induced p24 release even 60 minutes post KR13 incubation with HIV-1*

Conclusions

- The virolytic process by KR13 had a pattern of release of the viral components which is both concentration dependent and time dependent
- The breadth analysis showed that the cell-free KR13 induced p24 release is consistent across all clades of HIV-1 tested
- KR13 induced virolysis completely and irreversibly inactived the virion and retained active gp41
- T20 inhibition of virolysis showed that the 6 helix bundle formation of gp41 is important for the virolysis event
- HIV-1 R3A V38A T20 resistant mutant virus led to the loss of KR13 induced virolysis
- KR13 induced virolysis follows a similar timeline as a HIV-1–cell fusion
- *This therefore shows that KR13 might be hijacking the fusion machinery of the virus, inducing the virion to follow the Env transformation steps normally used for fusion even in absence of host cells*

Future Work

- Further explore the mechanism of virolysis by relating it to the HIV-1 fusion process
- Understand the physical nature of the virus post treatment with peptide triazole thiols by looking at its morphological signatures
- Test the breadth of virolysis among different clades of HIV-1

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