Role of Sulfhydryl in Causing Lytic Inactivation of HIV-1 by Env Targeting Peptide Triazole Thiols

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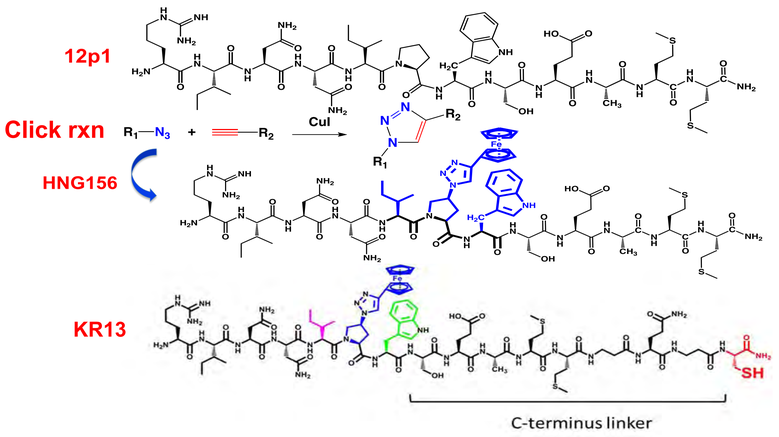
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Abstract

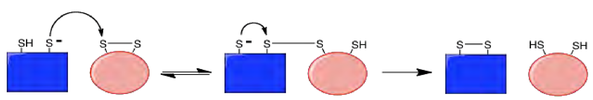
We have identified peptide triazole thiol inhibitors which bind Env gp120, allosterically inhibit CD4 receptor and co-receptor surrogate mAb 17b binding, and cause both gp120 shedding and p24 release from the virus lumen. The prototype peptide triazole thiol KR-13 (R-I-N-N-I-X-W-S-E-A-M-M-βA-Q-βA-C-CONH2, X= ferrocenyltriazole-Pro) causes p24 release, however KR-13b (C-terminal SH capped by acetomidomethyl) inhibited cell infection but did not cause p24 leakage (1). We are investigating the mode of action by which the C-terminal sulfhydryl group causes irreversible inactivation. From a molecular dynamics simulated model of the peptide triazole-gp120 complex we know that the IXW pharmacophore of KR13 binds in the CD4 binding site. Therefore, a working model is that the thiol interferes with conserved disulfides clustered proximal to the CD4 binding site in gp120 through "disulfide exchange". These disulfides are located within gp120 at 296-331 (V3 loop), 385-418 (C4), 378-445 (C3), and 119-205 (V1/V2 loop). To evaluate the importance of the spatial relationship between PT-SH and gp120 disulfide groups we synthesized truncated peptides derived from KR-13 and compared p24 release and antiviral activities. We observed a strong dependence of lysis activity on length of the linker between the IXW pharmacophore and SH group. We also examined the ability of KR13 to cause p24 release after HIV-1 treatment with Ellman's reagent, a disulfide exchange inhibitor. These results demonstrate that release of p24 induced by KR13 is significantly inhibited by Ellman's reagent. Additionally, we have shown that 2G12, a conformational antibody which binds to a glycosyl group near the V3 loop, competes with the p24 release of virolytic peptides in a dose dependent manner.

Background



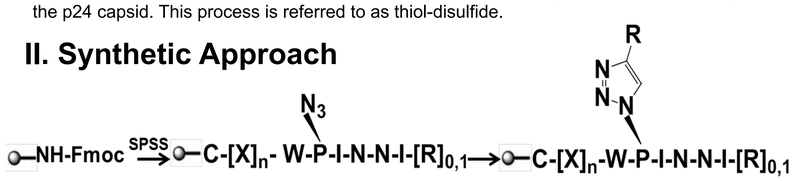
Synthetic Rationale

I. Rationale: Disulfide Exchange



In Thiol-Disulfide Exchange free cysteines disrupt naturally occurring disulfide bonds. We hypothesize that the C-terminal Cys of peptide triazoles binds to conserved disulfides of the HIV-1 envelope, thereby altering the structure of the membrane causing rupture and ultimately release of the p24 capsid. This process is referred to as thiol-disulfide.

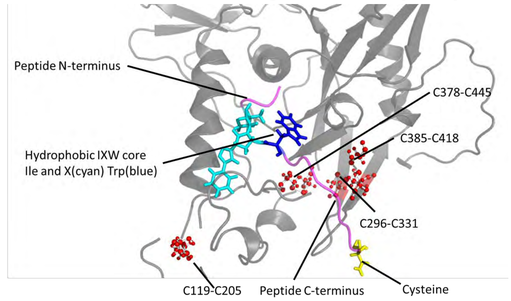
II. Synthetic Approach



KR13 showed novel cell-free virolytic inactivation of HIV-1. Both the core peptide triazole pharmacophore and C-terminal sulfhydryl group were found to be critical for the virolytic effect. We hypothesize that the cysteine engages in disulfide exchange with gp120 disulfides that surround the CD4 binding site. This process has been previously found to be is necessary for viral infection (2).

Results

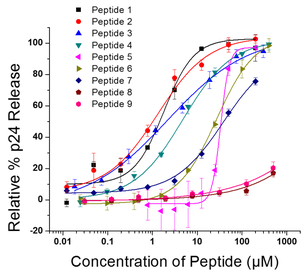
III. Rationale: Molecular Dynamics (MD) Simulation



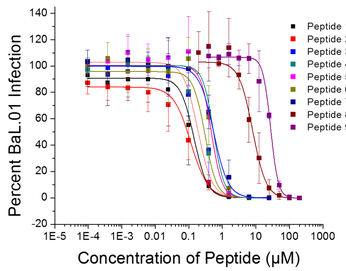
IV. Peptide Triazole Thiol Library Validation

Peptide Triazole Thiols	Sequence	Linker Atoms	W-SH Length (A°)	Antiviral (μM)	p24 Release (μM)	p24 EC50/ Antiviral EC50
KR13	RINNIXWS-E-A-M-M-βA-Q-βA-C	26	35.96	0.074 ± 0.0031	0.866 ± 0 .55	11.7
Peptide 1(HL01)	RINNIXWS-EA-βA-Q-βA-βA-C	24	38.99	0.143 ± 0.045	1.80 ± 0.9	12.6
Peptide 2(HL02)	RINNIXWS-EA-βA-Q-βA-C	20	33.14	0.114 ± 0.040	1.32 ± 0.26	11.6
Peptide 3(HL03)	RINNIXWS-EA-βA-Q-C	16	26.02	0.197 ± 0.038	2.20 ± 0.6	11.2
Peptide 4(HL04)	RINNIXWS-EA-βA-C	13	23.23	0.286 ± 0.013	4.76 ± 0.73	16.6
Peptide 5(LB05)	RINNIXWS-EA-C	9	18.70	0.469 ± 0.019	26.7 ± 0.8	56.9
Peptide 6(LB04)	RINNIXWS-E-C	6	14.52	0.554 ± 0.007	33.2 ± 0.9	59.9
Peptide 7(LB03)	RINNIXWS-C	3	10.07	0.550 ± 0. 063	38.4 ± 4.9	69.8
Peptide 8(LB02)	RINNIXW-C	0	5.75	7.03 ± 2.2	>> 500	>> 71.1
Peptide 9(LB01)	NNIXW-C	0	5.75	26.3 ± 0.78	>> 500	>> 19.0

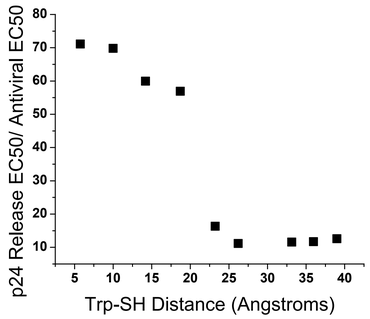
V. Virolysis (p24 Leakage)



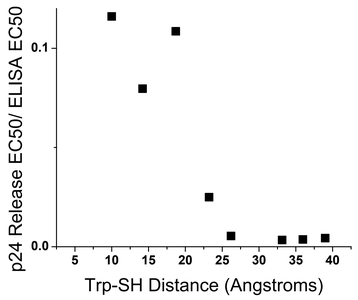
VI. Inhibition of Cell Infection



VII. Relative p24 Lysis vs. Distance (Based on Cell Infection)

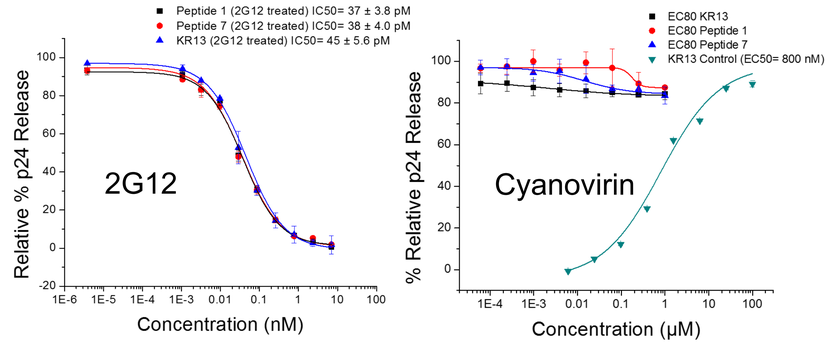


VIII. Relative p24 Lysis vs. Distance (Based on ELISA binding)



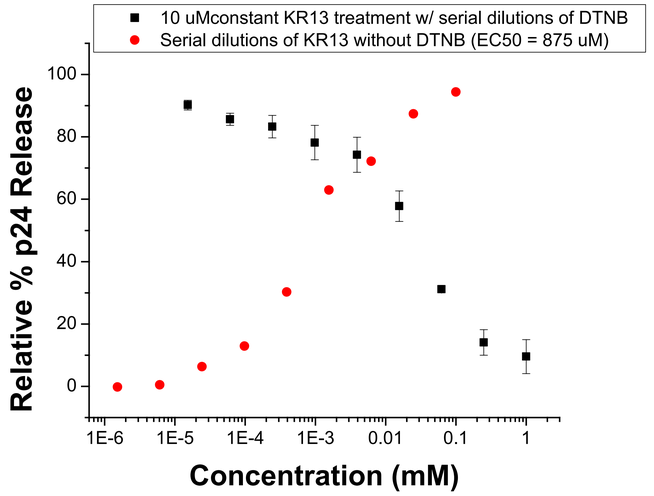
- p24 release efficiency is dependent on peptide C-terminus linker length.

IX. 2G12 Inhibits p24 Release of Virolytic Peptides



- Conformational lectin- binding antibody, 2G12, specifically inhibits virolysis.
- This implicates V3 loop disulfide C296-C331 involvement in HIV virolysis.

X. Ellman's Reagent (DTNB) Inhibits p24 Release



- DTNB inhibits p24 release in a dose dependent manner.

Conclusions and Future Directions

- There is a discontinuous relationship between virolysis and peptide length, indicating that a minimal length is required for efficient virolysis.
- The minimum linker length between PT pharmacophore and PT-SH appears needed to enable contact between SH and disulfides of the envelope protein.
- V3 loop disulfide is implicated in the disulfide exchange process.
- Our results support the hypothesis that thiol exchange occurs between the peptide C-terminal thiol group and a specific CD4 exposed disulfide cluster in gp120.
- Key goals going forward are to refine the pharmacophore, investigate the disulfide exchange process of virolysis and identify the specific Env protein disulfides involved.

References

1. Bastian et al., *ChemMedChem*. 2011, 2011 6: 1335-1339
2. Hugues et al. *PNAS*. 1994, 41: 4559-4563

Acknowledgement

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