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

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Abstract
We have identified peptide triazole thiol inhibitors which bind Env gp120, allosterically inhibit C4 receptor and co-receptor surrogates mAb 17b binding, and cause both gp120 shedding and p24 release from the virus lumen. The prototype peptide triazole thiol KR-13 (K-H-N-I-X-W-S-E-A-M-M-A-Q-Q-A-C-CNHS2). X: ferrocenyltriazole-Pro) causes p24 release, however KR-13b (C-terminal SH capped by acetylamidomethyl) inhibited cell infection but did not cause p24 release. 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 HIV-1 protease of KR-13 binds in the CD4 binding site. Therefore, a working model is that the thiol interferes with conserved disulfide clusters proximal to the CD4 binding site in gp120 through "disulfide exchange". These disulfides are located within gp120 at 299-331 (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 HIV-1 protease and SH group. We also examined the ability of KR-13 to cause p24 release after HIV-1 treatment with Elman's reagent, a disulfide exchange inhibitor. These results demonstrate that release of p24 induced by KR13 is significantly inhibited by Elman'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.

Results

I. Rationale: Disulfide Exchange

In Thio-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 thio-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 necessary for viral infection (2).

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 vaccine and PT-SH appears 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 thioc 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

2. Huguet et al., PNAS, 1994, 91: 4559-4563

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