PROBING THE DIMERIZATION OF THE HIV-1 5′ LEADER

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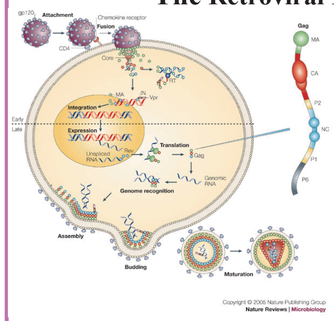
Abstract

Human immunodeficiency virus type-1 (HIV-1) infects roughly 35 million people worldwide. HIV-1 selectively packages the dimeric, unspliced RNA genome. It is important to characterize the dimerization mechanism of the HIV-1 5′-leader (5′-L) because it is the most conserved region of the genome and contains the major dimerization signal. Previous NMR studies from our lab have identified an extensive intermolecular dimer interface of the 5′-L after long periods of incubation in physiological conditions. Gel based studies of the 5′-L established the dimerization equilibrium to be approximately 30 minutes. However, the nature of the dimer is unknown. We used an NMR spectroscopy strategy known as long-range probing by Adenosine Interaction Detection (lr-AID) to probe the nature of the dimer interface at short time intervals. The two lr-AID mutations, UUA v. UUG, when introduced into the context of the full 5′-L, have distinct chemical shifts at 6.4 ppm and 6.7 ppm, respectively. By mixing A^d UUA 5′-L and A^H UUG 5′-L we were able to show that the 5′-L forms an extended dimer within 30 minutes, consistent with the gel-based studies. Therefore, extended dimer formation occurs on the same time scale as overall dimerization.

The Retroviral Life Cycle



The HIV-1 life cycle can be understood through two distinct stages: early and late phase. The early phase is characterized by viral infection of the host cell, reverse transcription of the viral genome, and integration in to the host DNA. The late phase is characterized by translation of the newly integrated genome, selection and packaging, virus assembly, and maturation.

Figure. 1. Life Cycle of HIV[1]

The HIV-1 Genome

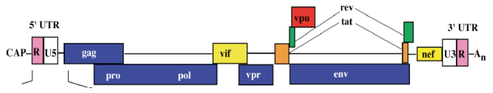


Figure 2. The 5′-untranslated region (5′-UTR) is located at the 5′ distal end of the HIV-1 genome.[2]

"Kissing" Dimer → Extended Dimer Equilibrium

HIV-1 genome selection is highly selective and only dimeric, unspliced genomes are recognized by Gag for packaging. The nature of dimer formation between viral RNA occurs in two proposed forms: a palindromic "kissing" interaction and an extended dimer conformation.

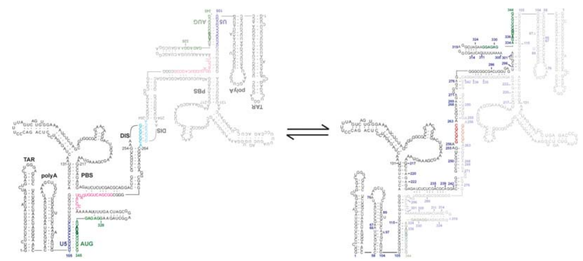


Figure 3. "Kissing" Interaction (*Left)* occurs at the DIS stem loop between palindromic GCGCGC sequences; this is a U5:AUG intramolecular interaction. The Extended Dimer Conformation (*Right)* retains the GCGCGC interaction; this is a U5:AUG intermolecular interaction

Long Range Probing by Adenosine Interaction Detection: (lr-AID)

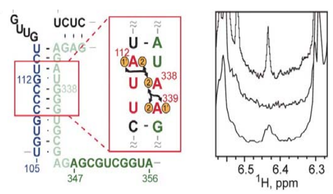


Figure 4. We are utilizing a sequence (UUA:UAA) found natively in the TAR stem loop of the 5′-L which exhibits a unique chemical shift in an isolated region of the NMR spectra in order to view the different conformations.[3] Previous studies show that when this sequence is introduced into the U5:AUG region of the 5′-L as a mutation, a similar peak is obtained.[3]

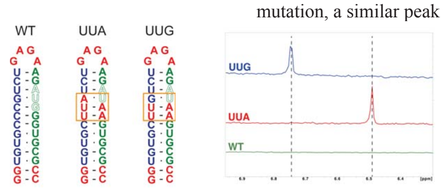
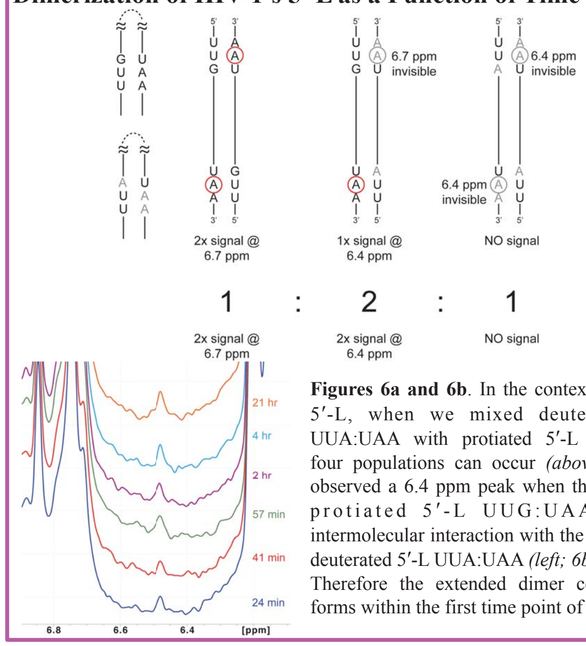


Figure 5. In order to monitor dimerization by NMR it is necessary to differentiate between the 5′ leaders involved. Our control hairpins indicated that the (UUA:UAA) and (UUG:UAA) sequences appeared at 6.4 and 6.7 ppm respectively. We will introduce these lr-AID mutations in the U5:AUG region of the full 5′-L to probe for kissing vs. extended conformations as a function of time.

Dimerization of HIV-1's 5′-L as a Function of Time by NMR



Figures 6a and 6b. In the context of the full 5′-L, when we mixed deuterated 5′-L UUA:UAA with protiated 5′-L UUG:UAA four populations can occur *(above; 6a*). We observed a 6.4 ppm peak when the U5 of the protiated 5′-L UUG:UAA has an intermolecular interaction with the AUG of the deuterated 5′-L UUA:UAA *(left; 6b)*.
Therefore the extended dimer conformation forms within the first time point of 24 minutes.

Dimerization of HIV-1's 5′-L as a Function of Time by Gel Electrophoresis

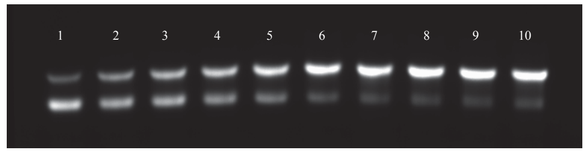


Figure 7. This is a 1% TB gel showing the dimerization of TSL4-Native Blunt in PI Buffer (20mM Tris, 140mM KCl, 10mM NaCl, and 1mM $MgCl_2$) at different time points . Lanes are as follows; **1.** 0 min, **2.** 0.5 min, **3.** 1 min, **4.** 2 min, **5.** 5 min, **6.** 10 min, **7.** 30 min, **8.** 60 min, **9.** 120 min, **10.** 24 hr.

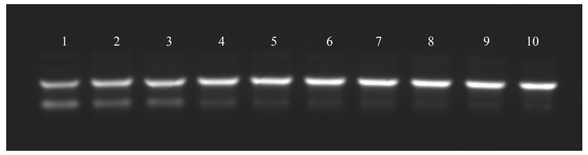


Figure 8. This is a 1% TB gel showing the dimerization of TSL4-344 in PI Buffer (20mM Tris, 140mM KCl, 10mM NaCl, and 1mM $MgCl_2$) at different time points . Lanes are as follows; **1.** 0 min, **2.** 0.5 min, **3.** 1 min, **4.** 2 min, **5.** 5 min, **6.** 10 min, **7.** 30 min, **8.** 60 min, **9.** 120 min, **10.** 24 hr.

Conclusion/ Future Work

Through this NMR study and time-dependence gel studies, we were able to establish that the extended dimer of the 5′-L forms within 30 minutes. Our next step is to characterize the differences in RNA binding between the GAG polyprotein, capsid-nucelocapsid (CANC), and nucleocapsid (NC).

Acknowledgements

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References

[1] B. G. Turner, M. F. Summers, *J. Mol. Biol.* (1999) 285, 1 – 32

[2]Lu, K., Heng, X., Garyu, L., Monti, S., Garcia, E. L., Kharytonchyk, S., ... & Summers, M. F. (2011). *Science*, *334*(6053), 242-245.

[3] Kun Lu *et al. Science* 334, 242 (2011)