Conformational Dynamics of the Src-family Kinase Hck upon HIV-1 Nef and Small Molecule Inhibitor Binding

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OUTLINE

OBJECTIVE

Determine conformational changes in the Src-family kinase Hck upon activation by HIV Nef and inhibition by the anti-retroviral compound DFP-4AB

METHODS

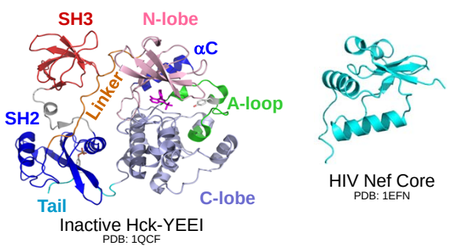
Hydrogen exchange mass spectrometry

RESULTS

Nef association induces minor changes in Hck, which are reversed in the presence of DFP-4AB

INTRODUCTION

Hck, a Src-family kinase, is activated by the HIV-1 accessory protein Nef (1). Nef binds to the SH3 domain of Hck, resulting in displacement of an intramolecular interaction with the SH2-kinase linker. Nef-mediated SH3 domain displacement results in constitutive Hck activation, an interaction implicated in HIV pathogenesis (2). A high-throughput screening campaign targeting the Nef:Hck complex led to the discovery of the small molecule kinase inhibitor DFP-4AB, which selectively inhibits Nef-dependent Hck activity in biochemical assays and potently blocks HIV replication in vitro (3). Hydrogen exchange mass spectrometry (HX MS) was used to study conformational changes in Hck that result from Nef binding, as well as the effect of DFP-4AB binding on these changes.

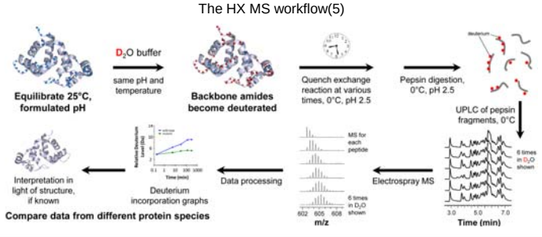


METHODS

Recombinant, downregulated Hck (Hck-YEEI) and HIV Nef were produced as 6x-His-tagged constructs in Sf9 insect cells and *E.coli*, respectively. Hck-YEEI was purified by anion exchange chromatography followed by immobilized metal-ion affinity chromatography (IMAC). Nef was purified by IMAC. DFP-4AB was synthesized as described (3).

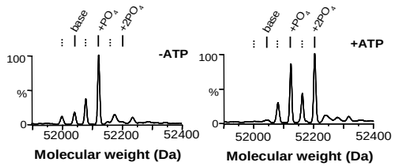
Continuous labeling hydrogen exchange experiments were conducted at room temperature. Immediately following quench, samples were injected into a nanoACQUITY with HDX technology for online pepsin digestion, followed by UPLC reversed-phase separation (4). Mass analysis was performed on a WATERS SYNAPT G1 mass spectrometer with an ESI source. Peptides were identified using PLGS 2.5 and deuterium incorporation data were analyzed with DynamX 2.0 (Waters).

The HX MS workflow(5)



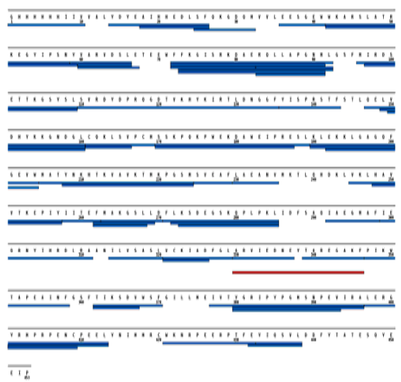
RESULTS AND DISCUSSION

Hck-YEEI Phosphorylation Status



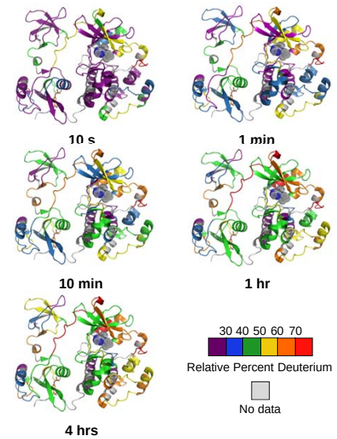
Electrospray mass spectra of intact Hck-YEEI before (-ATP) and after (+ATP) autophosphorylation. Lines are shown above the spectrum to indicate the mass of acetylated (base, +42 Da) and phosphorylated forms (+80 Da per phosphate). The -ATP sample is phosphorylated on the tail tyrosine while the +ATP sample is phosphorylated on both the tail and activation loop tyrosines.

Hck-YEEI Peptide Coverage Map



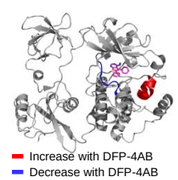
Map of Hck-YEEI peptic peptides identified and followed during HX MS experiments. 86% of the linear sequence is covered. The red peptide was found only in the +ATP samples.

Hck-YEEI Baseline Exchange



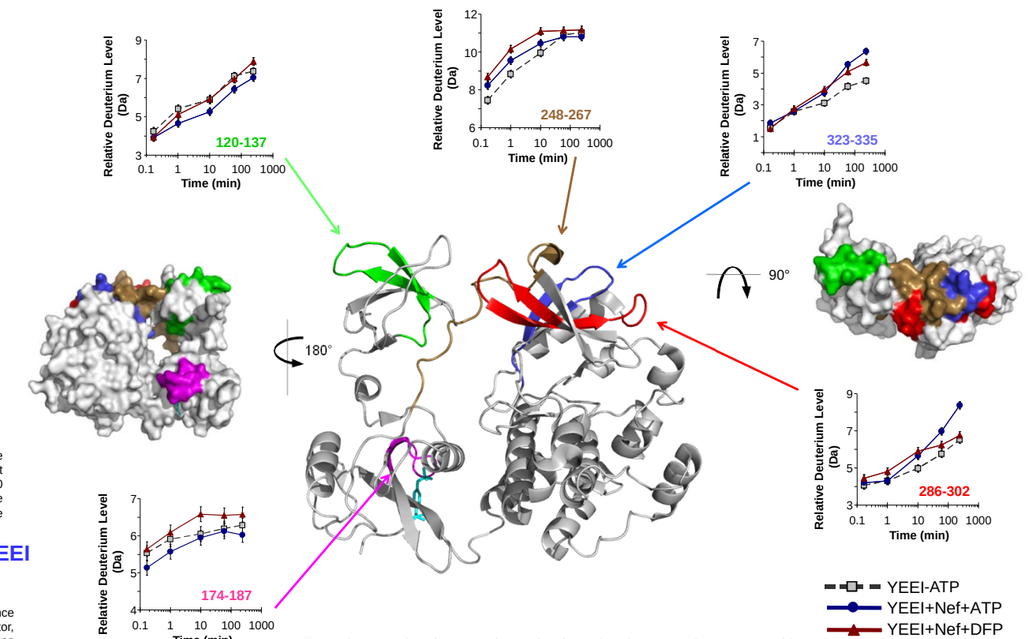
Baseline deuterium incorporation is illustrated on the ribbon structure of Hck-YEEI (PDB: 1QCF). Data are presented as relative percent deuterium incorporation using a six color scale at 10 sec, 1 min, 10 min, 1 hour and 4 hours of exchange in D_2O buffer. Regions of the protein without peptide data are shown in gray. The small molecule inhibitor PP1 (shown as spheres) marks the active site.

DFP-4AB-induced changes in Hck-YEEI



Deuterium incorporation in the presence of the small molecule kinase inhibitor, DFP-4AB. A one dalton change was observed in the regions indicated on the structure of Hck, where the compound has been modeled into the binding site. No significant changes were observed in the presence of ATP.

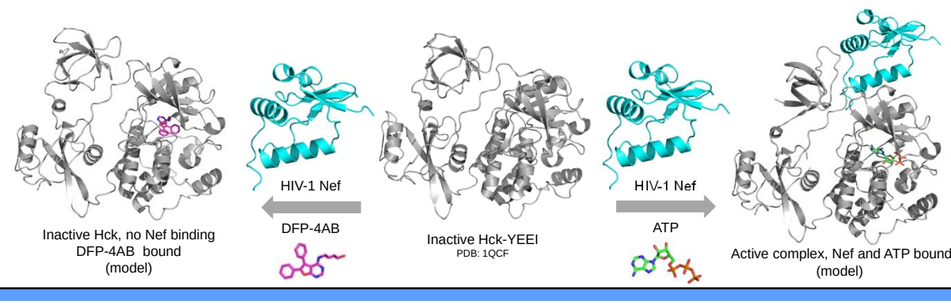
Changes in activated and inhibited Hck-YEEI when bound to Nef



Deuterium uptake plots are shown for deuterium incorporation measured in Hck-YEEI alone (grey squares) and in the presence of either ATP (blue circles) or DFP-4AB (red triangles). Peptides are mapped onto the Hck-YEEI structure by the corresponding color of their sequence numbering.

CONCLUSIONS

- Nef binding does not result in global conformational changes to Hck despite kinase activation
- The Hck SH2 domain remains engaged with the pTyr-tail when Hck is bound to Nef and in the presence of ATP
- The SH3 domain binds to Nef in the presence of ATP and to either Nef or the SH2-Kinase linker in the presence of DFP-4AB
- Changes in the linker and N-lobe of the kinase domain are observed in the presence of Nef and ATP, but reversed in the presence of Nef and DFP-4AB, suggesting that this compound reverses Nef-dependent changes to the active site or that Nef may not bind in the presence of inhibitor



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REFERENCES

1. Saksela, K., *et al.* (1995). *EMBO J.* **14**, 484-91.
2. Hanna, Z., *et al.* (2001). *J. Virol.* **75**, 9378-92.
3. Emert-Sedlak, L., *et al.* (2009). *ACS Chem Biol.* **4**, 939-47.
4. Wales, T.E., *et al.*, (2008) *Anal. Chem.* **80**, 6815-20.
5. Engen, J.R. *et al.*, (2011) *Ency. Anal. Chem.*, ISBN 978047002731.