SUICIDE INHIBITION OF ONCOGENIC K-RAS G12C PROCEEDS VIA SHIFT TO THE INACTIVE CONFORMATION

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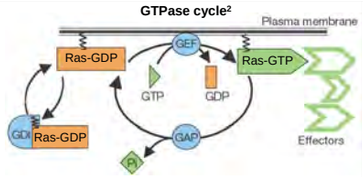
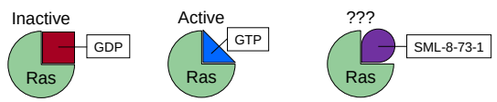
INTRODUCTION

Ras proteins operate as molecular switches in the cellular signaling network and are often activated by oncogenic mutations that change their activity.[1]

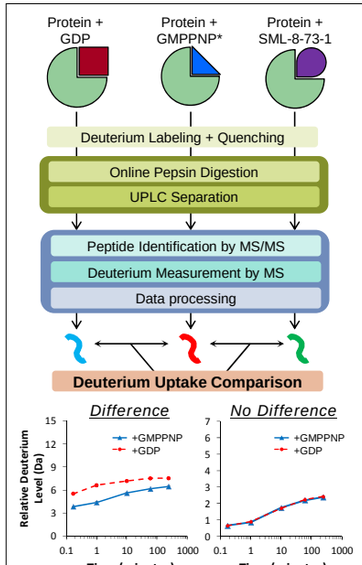


To date, there are no commercial therapeutics targeting Ras, yet oncogenic Ras proteins are prevalent in 20-25% of all tumor types.[3] GDP-analogues have been synthesized which irreversibly bind to the mutant cysteine in the G12C mutant of K-Ras, a variant which occurs in 10-20% of all Ras-driven cancers.

The goal of this study was to use HX MS to understand how covalent binding to K-Ras G12C by the GDP-analogue SML-8-73-1 altered the protein conformation. Does it push the protein into an inactive conformation similar to its GDP-bound state, or does it push the protein into an active conformation similar to its GTP-bound state?



METHODS



K-Ras G12C mutant previously incubated with GDP, a non-hydrolyzable GTP mimic (*GMPPNP), or SML-8-73-1 was independently labeled with deuterium at room temperature using the same experimental conditions.[4] Mass spectral analyses were performed with a Waters Q-Tof Premier equipped with a standard ESI source. Online pepsin digestion was performed to generate peptic peptides, which were identified in undeuterated samples with Waters MS^E and Waters PLGS 2.5. Exchange into the three forms was compared using DynamX software. Workflow schematic shown at left.[5]

RESULTS AND DISCUSSION

Figure 1 – K-Ras G12C Peptide Map (Online Pepsin Digestion)

Generates over 40 unique peptides, 94% coverage (created using MSTools[6])

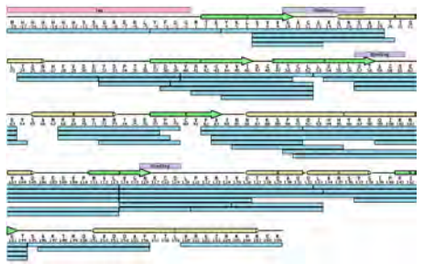
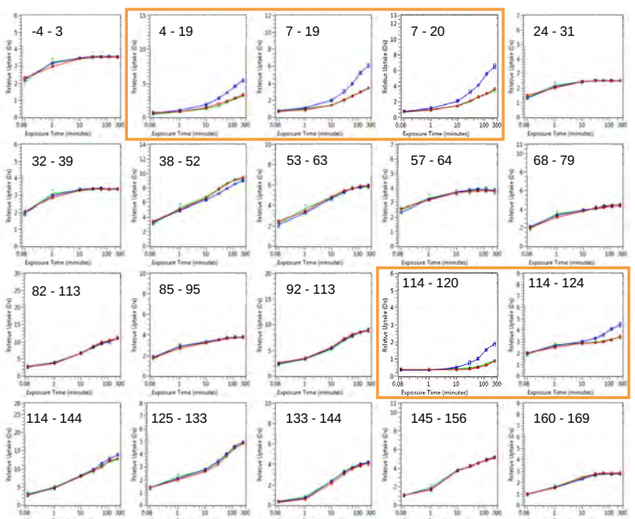


Figure 2 – Relative Deuterium Uptake Plots

Comparison between GDP-bound, GMPPNP-bound, and inhibitor-bound states



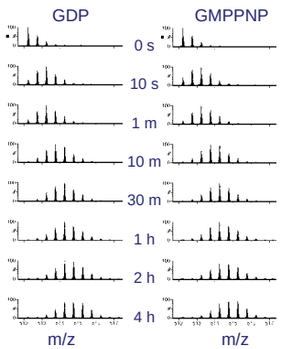
GMPPNP GDP SML-8-73-1

> 0.5 Da difference between states

- No difference in HX between states were observed in most of protein
- Several peptides showed differences:
  - The inhibitor-bound state mirrors GDP-bound (inactive) state; Green and red lines are on top of one another

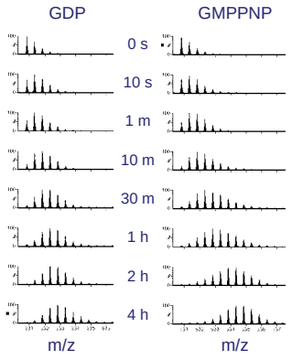
Figure 3 – Some Regions Exhibit EX1 Kinetics, Most Regions Do Not

Residues 83-91 (representing most of protein): EX2 Kinetics



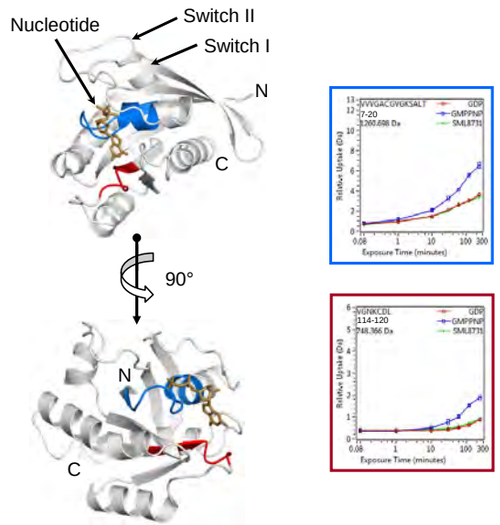
- No broadening of isotopic distribution regardless of bound state
- Most peptides in this protein exhibit EX2 kinetics

Residues 7-20 (VVVGACGVGKSALT): EX1 Kinetics



- Isotopic distribution for this peptide indicates heterogeneous populations when the protein is in the active state
- This phenomenon is unusual and indicates significant protein dynamics in the region covering residues 7-20 of K-Ras G12C

Figure 4 – Key Regions of Difference Between States



> 2.0 Da difference between states

0.5 – 2.0 Da difference between states

< 0.5 Da difference between states

CONCLUSIONS

- **Residues 7-20: adjacent to phosphate groups, significantly higher deuterium uptake in active conformation;**
- **Residues 114-120: adjacent to guanosine moiety, slightly higher deuterium uptake in active conformation**
- **Rest of protein: no significant difference in deuterium uptake between all states**
- **When bound to covalent inhibitor SML-8-73-1, deuterium uptake of K-Ras G12C mirrors GDP-bound state**
- **SML-8-73-1 likely stabilizes an inactive form of the protein and may deactivate oncogenic signaling**
- **Covalent inhibition may provide a viable means of targeting Ras directly, which has not been done successfully to date**
- **Conformational perturbations in proteins driven by small molecules are difficult to measure by most methods but can be easily interrogated using HX MS**

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