Intracellular Creatine Sensing and Creatine Homeostasis

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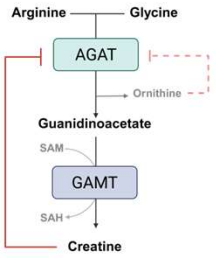
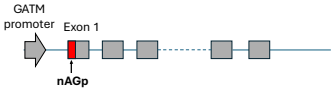
Background



Creatine Synthesis Regulation

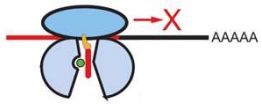
- Creatine synthesis is regulated in two ways
  - Competitive inhibition by ornithine
  - Negative feedback loop by creatine



- A portion at the beginning of the AGAT protein (nAGp) is essential for the creatine synthesis feedback loop

Hypothesis

Creatine downregulates AGAT expression by interacting with the nAGp in the ribosomal exit tunnel causing ribosomal stalling.



Methods

Nuclear Magnetic Resonance (NMR) Spectroscopy

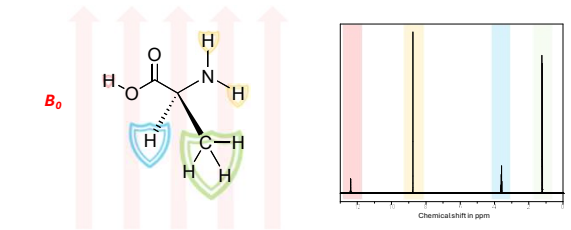


Figure 1. Overview NMR Spectroscopy. On the left is an example molecule in a magnetic field (B_0). On the right is the example of the NMR spectrum for this molecule.

- Detects change in chemical/magnetic environment of NMR active nuclei (^1H, ^{15}N, etc.)
- The different chemical environments of the hydrogen nuclei result in different levels of shielding from the magnetic field
- Different levels of shielding translate to different chemical shifts in the NMR spectrum
- Can use NMR to look at just H^1 or H^1 and N^{15} together

Methods

NMR Spectroscopy (cont'd)

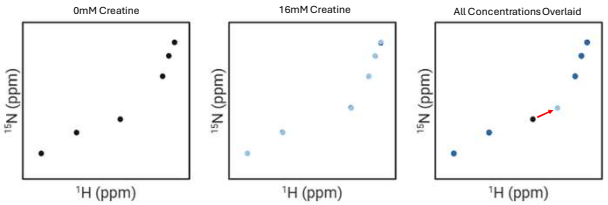
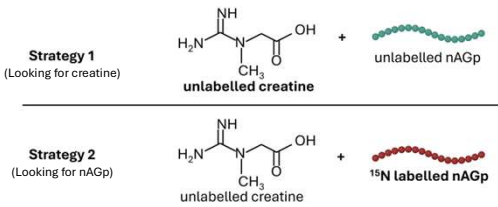


Figure 2. 2D NMR Spectroscopy Overview. Example spectra showing an interaction between creatine and the molecule under investigation.

- 2D NMR spectra put the ^{15}N and ^1H spectra one on each axis and combine the peaks to form dots in the graph
- These dots represent the chemical shifts of these ^{15}N-^1H pairs
- If creatine interacts with the molecule under investigation some of the dots would move

Investigation Strategies



Results

nAGp Does Not Interact with Creatine Alone

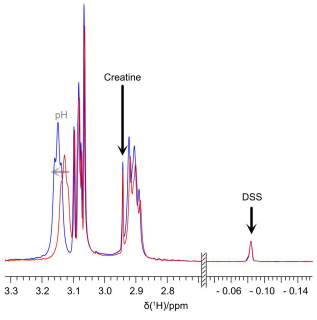


Figure 3. H^1 NMR Spectrum of nAGp and Creatine. NMR spectrum from 1mM creatine alone, shown in red. NMR spectrum from 1mM creatine mixed with peptide, shown in blue. If an interaction occurs, the creatine peak would be lower in the blue spectrum. Since this is not the case, this indicates that creatine and nAGp do not interact alone. The slight differences between spectra are likely due to a change in pH between the samples, indicated by the grey arrow.

Results

nAGp Does Not Interact with Creatine Alone

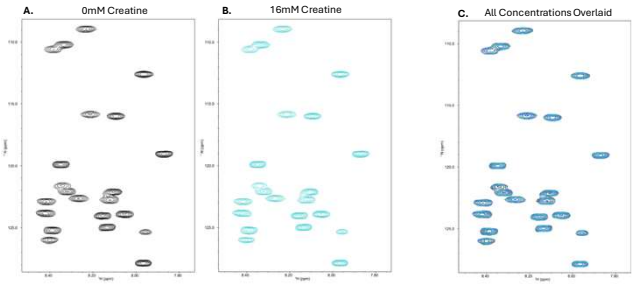
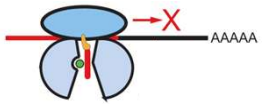


Figure 2. 2D NMR Spectrum of nAGp and Creatine. A. NMR spectrum of peptide alone (0mM creatine added). **B.** NMR spectrum with the maximum amount of creatine added (16mM). **C.** NMR spectra from all concentrations overlaid. The spectra are identical, indicating that creatine and nAGp do not interact without other factors present.

Conclusions & Future Directions

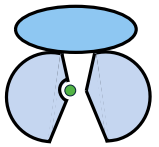
Conclusions

- nAGp does not interact with creatine alone
- Aligns with hypothesis that ribosome is needed for this interaction



Future Directions

- Does creatine interact with the ribosome without other factors present?
- This work will better our understanding of how creatine synthesis is regulated
- This will help develop therapeutics which can enhance or mute AGAT's response to creatine



Acknowledgements

I would like to thank Dr. Schulze and all the members of the Schulze lab for their support in this project. I would also like to thank Dr. Philip Roessler from the Kay lab for his help and NMR expertise. Finally, I would like to thank ACD for their support and advice on this project.