The Effect of Lanthionine Ketimine on CRMP1's Ability to Control Actin Assembly

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INTRODUCTION

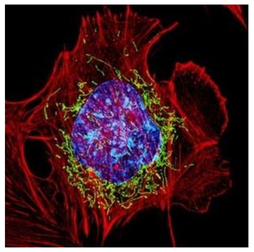
The actin cytoskeleton is an essential component for cell shape and function. Yet our understanding of how cells control actin assembly is still limited. Studies have found that a family of proteins, known as Collapsin Response Mediator Proteins (CRMPs), control the actin cytoskeleton network in various cell processes. A member of this family, CRMP1, has been found to play a role in axonogenesis, cancer cell metastasis suppression, and the regulation of cell migration and cell-cell adhesion in kidney epithelial cells. In addition, a small molecule naturally abundant in the human brain, lanthionine ketimine (LK), has been found to directly bind to CRMP2, but this binding interaction has not been quantified and its physiological significance is not yet known. Based on ~75% shared sequence identity of CRMP1 and CRMP2, LK likely interacts with CRMP1 and influences the ability of CRMP1 to regulate actin assembly. Now, the binding interaction between CRMP1 and LK is discovered and quantified, and a maximal binding of LK is found to increase the interaction between CRMP1 and filamentous actin (F-actin).

AIMS

1. **Determine if LK binds to CRMP1**
2. **Demonstrate if LK affects CRMP's binding to F-actin**

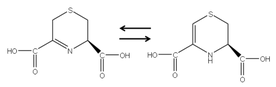
BACKGROUND

The Actin Cytoskeleton

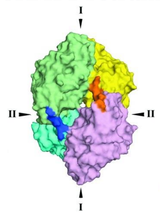


An Actin Filament

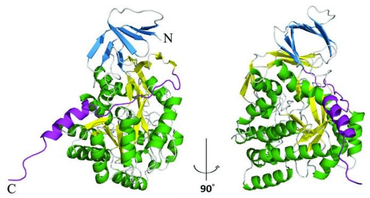
Lanthionine Ketimine



CRMP1 Tetramer



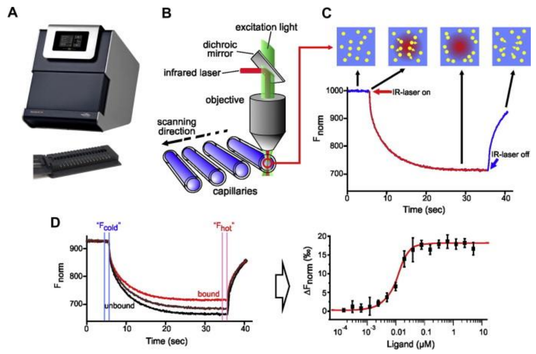
CRMP1 Monomer



METHOD

Aim 1. MicroScale Thermophoresis (MST)

A biomolecular interaction quantification technique based on thermophoresis, the oriented movement of molecules in a temperature gradient.

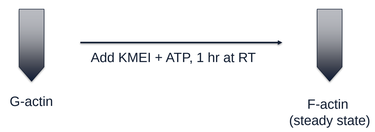


To see if the unstructured tail plays a role in binding, the binding interaction between LK and full-length CRMP1 vs. truncated CRMP1 are measured using MST.

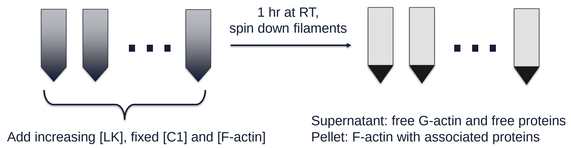
Aim 2. Actin Co-sedimentation Assay

An in vitro assay to analyze specific proteins binding to F-actin. In this experiment, a serial change in LK's concentration is applied; the concentration of CRMP1 and F-actin are held the same. Protein gel electrophoresis is used to visualize the result.

1) Actin Polymerization



2) Incubation with CRMP1 and LK



3) Gel Electrophoresis

Run pellet and supernatant separately on a gel, allowing visualization of the amount of bound protein (in the pellet) vs unbound protein (in the supernatant).

RESULTS & ANALYSIS

Aim 1

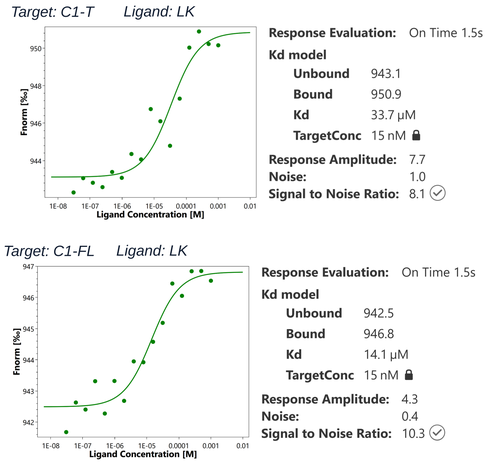


Figure 1. Both pictures are examples of our MST results. (1a) Upper graph. CRMP1 without its unstructured tail binds to LK at a Kd of ~33.7 μM. (1b) Lower graph. Full-length CRMP1 binds to LK at a Kd of ~14.1 μM.

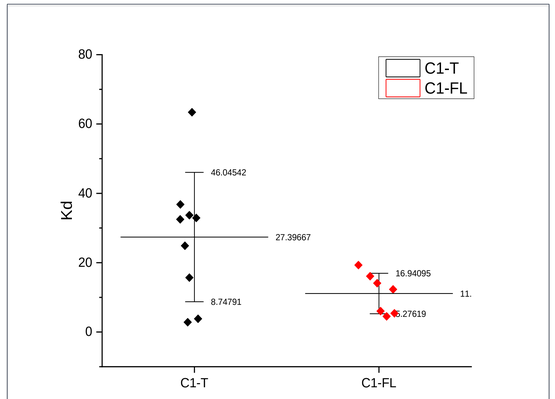


Figure 2. Statistical analysis of all MST results. Each box plot shows a mean value and two values equal to mean +/- 1 SD.

Results show that full-length CRMP1 binds to LK more tightly than truncated CRMP1.

Possible explanations:
1) The unstructured C-terminus tail directly facilitates binding interaction.
2) The presence of the tail change the stoichiometry of the core structure of CRMP1 to a more favorable state, allowing tighter binding.
3) The presence of this tail allows a more opened contact between CRMP1 monomers in its tetramer state, which contributes to a tighter interaction with LK.

Aim 2

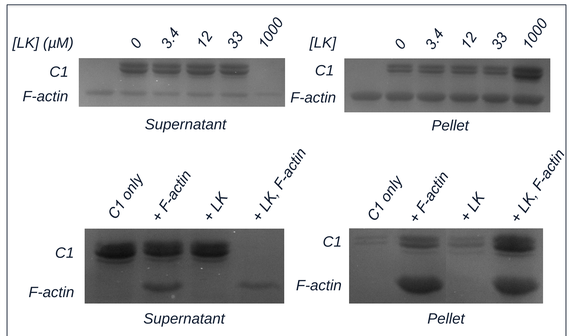


Figure 3. Actin co-sedimentation results. [C1] and [F-actin] are the same in both experiments. (3a) Upper two pictures. [LK] variation as shown. (3b) Lower two pictures. Control experiment. [LK] kept at 1000 μM.

A maximal binding of LK is sufficient to strengthen the binding interaction between CRMP1 and F-actin.

Possible explanations:
1) A saturation binding of LK is needed for the CRMP1 tetramer to have a change in its binding affinity with F-actin.
2) A less favorable tautomeric form of LK is preferred by CRMP1, so an even higher concentration of LK should be reached for saturation binding to occur.

FUTURE DIRECTIONS

Continue exploring the effect of LK binding to CRMP1 on the interaction between CRMP1 and F-actin.
- Determine the minimal concentration of LK needed to affect CRMP1's binding interaction with F-actin, by actin co-sedimentation assay with increasing LK concentration of 30-1000 μM
- Quantify binding interaction between F-actin and CRMP1 in the presence of maximal binding of LK, and compare the quantification with baseline binding interaction

Investigate the effect of LK on CRMP1's ability to control actin assembly

Find out the role of CRMP1's tail on binding interaction with LK

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ILLINOIS