Investigating the Interactions Between Individual Calmodulin and HIV-1 Protein Domains

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**Introduction**

HIV. A virus that weakens the immune system through viral replication and the destruction of CD4+ T-cells, which are white blood cells that detect infection and make antibodies. A cure for HIV has not yet been discovered.

MA. A matrix protein peptide found on the Gag polyprotein of HIV-1 which assists in regulating the stages of viral replication. MA contains two tryptophan-containing helices on both its N-term and C-term domains. Studies show that the myristoyl group of MA forms a complex with CaM.

CaM. A multifunctional calcium-binding regulatory protein in the human body. Calmodulin (CaM) has been found to be upregulated upon HIV infection. The MA/CaM complex induces extended conformation and causes a decrease in the compact structure of MA, which is predicted to impact the accessibility of interaction sites within MA and lead to rapid HIV viral production.

**OBJECTIVE.** Through hindering the myristoyl group on MA, the production of HIV may greatly decrease. Identifying how each protein domain is involved enhances current understanding of HIV production and is a significant step in determining a possible solution for inhibiting HIV-1 replication.

![MA:CaM Complex Structure](image)

**Methods**

**Fluorescence Spectroscopy** - The shifts and quenching of intensity peaks caused by the fluorescing interactions were analyzed at an excitation wavelength of 290nm. Titration of CaM were performed incrementally into MA.

**Anisotropy** – The change in orientation of the protein complex was analyzed using 0° and 90° polarized light. One polarizer was placed in excitation light path (0°) and the other in the emission light path (0° or 90°) to calculate anisotropy.

![Figure 2. Fluorescence Spectroscopy-Anisotropy Technique](image)

**Results – Fluorescence Spectroscopy**

**MA Domains – Apo and Ca^2+** Containing Conditions

**Findings.** Quenching occurs in all plots, but most significantly in the C-term plots (complete quenching). This suggests that the C-term CaM domain is either internally or externally quenched. More trials are needed to identify the exact cause of quenching. The N-term CaM domain appears to bind to MA, but does not experience a change in conformation in apo conditions.

**CaM Domains – Apo Conditions – Anisotropy vs Wavelength**

**Findings.** The anisotropy results in apo conditions demonstrate that the N-term CaM experiences a change in molecular orientation that suggests that molecular rotation is slowing down, which is a result of complex formation. The C-term CaM may also experience a change in orientation, however, there is too much noise in the data for proper conclusions to be drawn yet.

**Results – Anisotropy**

**CaM Domains – Apo Conditions – Intensity vs Wavelength**

**Findings.** Quenching occurs in all plots, but most significantly in the C-term plots (complete quenching). This suggests that the C-term CaM domain is either internally or externally quenched. More trials are needed to identify the exact cause of quenching. The N-term CaM domain appears to bind to MA, but does not experience a change in conformation in apo conditions.

**CaM Domains – Apo Conditions – Anisotropy vs [CaM]**

**Findings.** The anisotropy results in apo conditions demonstrate that the N-term CaM experiences a change in molecular orientation that suggests that molecular rotation is slowing down, which is a result of complex formation. The C-term CaM may also experience a change in orientation, however, there is too much noise in the data for proper conclusions to be drawn yet.

**Summary and Conclusions**

1. The blue shift of N-term MA in Ca^{2+} conditions indicates conformational change and suggests that the N-term MA binds CaM prior to the C-term MA.
2. Complete quenching of the C-term CaM intensity signal in apo conditions indicates external or internal Trp quenching may occur.
3. Quenching of the N-term CaM intensity signal in apo conditions and its associated anisotropy plot indicates N-term CaM binds in apo conditions, but does not involve a change in conformation.

**Objective**

To investigate each protein domain and tryptophan signal separately in Ca^{2+} containing and deprived conditions so that the location where binding occurs can be isolated and it can be determined if the interaction of one CaM or MA domain is required, or a prerequisite, for the other.

**Materials**

- N-term, C-term, Full CaM (50uM)
- N-term, C-term, Full MA (1uM)
- Buffer (Ca^{2+} containing)
- Apo Buffer (Ca^{2+} deprived) Mimic Physiological Conditions

**References**