Fall 11-30-2022

Analyzing Interactions of Calmodulin with HIV-1 Matrix Protein

Andrea Sandoval
Chapman University, ansandoval@chapman.edu

D. Mau
Chapman University

N. Karimi
Chapman University

K. Sakamaki
Chapman University

C. Owens
Chapman University

See next page for additional authors

Analyzing Interactions of Calmodulin with HIV-1 Matrix Protein

Abstract

Human immunodeficiency virus (HIV) attacks the immune system and if left untreated, could cause acquired immunodeficiency syndrome (AIDS). The HIV matrix protein (HIV-MA) is involved in replication and regulation of the HIV virus. Calmodulin (CaM), a calcium-binding protein found in all eukaryotes, has a potential role in the viral replication of HIV-MA which plays a key role in the replication of HIV. In order to investigate the interactions between calmodulin and the HIV-MA, a series of titrations with CaM are performed using circular dichroism. Circular dichroism (CD) uses circularly polarized light to observe the secondary structure of a molecule. The circularly polarized light is broken up into left and right components. When the molecule contains a chiral center, the left and right components are absorbed to different extents, and the differential absorption is measured with CD. Through a series of titrations, the chemical environment is changed in small increments so the molecule will experience conformational changes. As the conformation changes, CD is used to measure the ellipticity which provides a better understanding of the secondary structure that is a result of these chemical interactions. Since CaM plays a potential role in the viral replication of HIV-MA, CD is used to investigate the protein-protein interactions and conformational changes.

Keywords

HIV, Calmodulin, Fluorescence Spectroscopy, Titration

Disciplines

Biochemistry

Authors

Andrea Sandoval, D. Mau, N. Karimi, K. Sakamaki, C. Owens, and Jerry LaRue

Analyzing Interactions of Calmodulin with HIV-1 Matrix Protein

Andrea Sandoval, D. Mau, N. Karimi, K. Sakamaki, C. Owens, J. LaRue

LaRue CatLab Chapman University

Research Question: How does the presence of calcium affect the interaction between calmodulin and the HIV-1 matrix protein?

Introduction:

Human immunodeficiency virus (HIV) attacks the immune system, and if left untreated, could cause acquired immunodeficiency syndrome (AIDS). The HIV matrix protein is involved in the replication and regulation of the HIV virus. Calmodulin, a calcium-binding protein, has a potential role in the viral replication of the HIV matrix protein. In order to investigate the interactions between calmodulin and the HIV-1 matrix protein, a series of titrations are performed using circular dichroism.

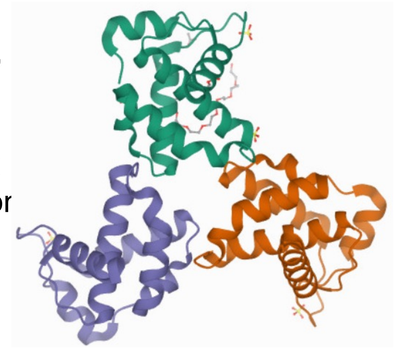

Figure 1: Structure of HIV-MA protein (Protein Data Bank)

Calmodulin and HIV-1 MA:

The HIV-1 matrix protein (HIV-MA), shown in Figure 1, plays a crucial role in regulating and replicating the HIV virus.

Calmodulin (CaM), shown in Figure 2, is a calcium-binding protein expressed in all eukaryotic cells. CaM is activated upon the binding of calcium, allowing for binding to other proteins, such as HIV-MA.

CaM contains a C-terminal and an N-terminal which are the functional groups on the ends of amino acids where binding occurs.

Previous studies have shown that the binding of calmodulin causes conformational changes to the HIV-1 matrix protein. CaM is upregulated upon HIV infection and has a potential role in the viral replication of the HIV matrix protein.

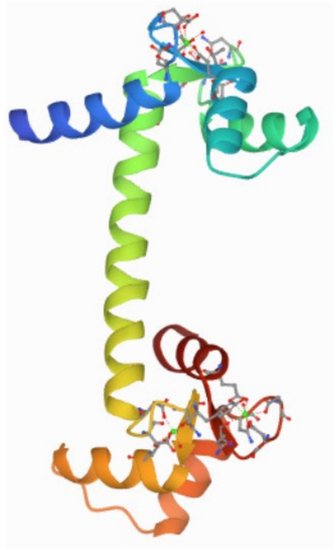

Figure 2: Structure of Calmodulin protein (Protein Data Bank)

Fluorescence Spectroscopy:

Fluorescence anisotropy spectroscopy uses horizontally and vertically polarized light to observe the orientation of the molecule and changes in the chemical environment. HIV-MA is not naturally fluorescent, so it is tagged with fluorescein, allowing for light to be emitted at the molecule. The protein with fluorescein is excited at 480nm, and the wavelength that is emitted is observed. By using different combinations of the polarizers, the rotational information of the molecule can be observed. Through a series of titrations, the chemical environment is changed in small increments so that the molecule will experience conformational changes. Since calcium is needed for the activation of CaM, the molecule will be observed under apo conditions, which indicates a lack of calcium, and in the presence of calcium. HIV-MA serves as the titrant and is titrated with CaM for apo conditions and CaCl for calcium conditions. Based on the conditions that the proteins are in, the binding of the C-terminal, N-terminal, and full calmodulin protein to HIV-MA are impacted.

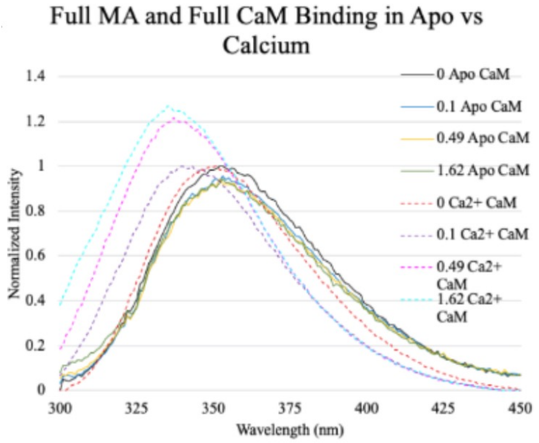

Figure 3: Fluorescence curves of HIV-MA protein binding to full CaM in apo and calcium conditions while increasing the concentration of CaM.

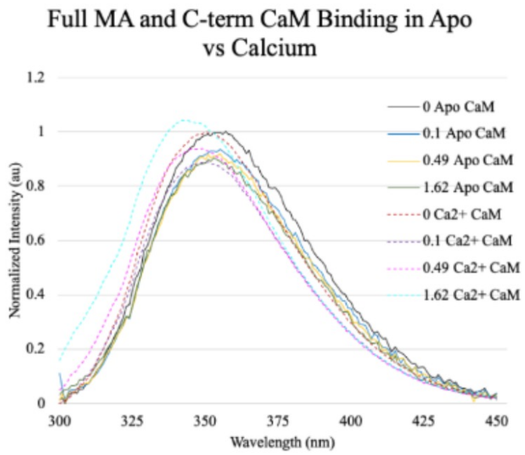

Figure 4: Fluorescence curves of HIV-MA protein binding to C-terminal CaM in apo and calcium conditions while increasing the concentration of CaM.

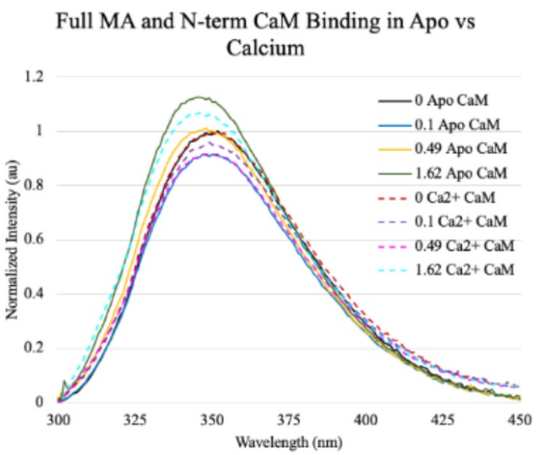

Figure 5: Fluorescence curves of HIV-MA protein binding to N-terminal CaM in apo and calcium conditions while increasing the concentration of CaM.

Conclusion:

CaM plays a potential role in the viral replication of HIV-MA, which plays a key role in the replication of HIV. Circular dichroism is used to investigate the protein-protein interactions and conformational changes. By gaining a better understanding of these interactions, we can begin to understand the role that CaM plays in HIV replication.

References:

(1) HIV Basics | HIV/AIDS | CDC. 2020 Nov 3. https://www.cdc.gov/hiv/basics/index.html.
(2) Chin D, Means AR. 2000. 10(8):322–328. doi:10.1016/S0962-8924(00)01800-6.
(3) Akyol Z, Bartos JA, Merrill MA, Faga LA, Jaren OR, Shea MA, Hell JW. 2004. J Biol Chem. 279(3):2166–2175. doi:10.1074/jbc.M302542200.
(4) Kelly, S. M.; Jess, T. J.; Price, N. C. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics 2005, 1751 (2), 119–139. https://doi.org/10.1016/j.bbapap.2005.06.005.