OBSERVING THE EFFECTS OF pH ON THE FELINE IMMUNODEFICIENCY VIRUS MATRIX PROTEIN MYRISTYL SWITCH

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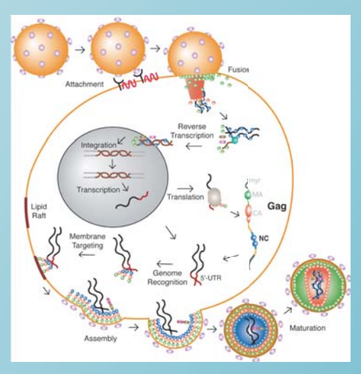
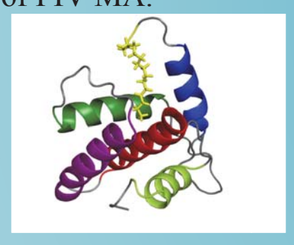
Abstract

The feline immunodeficiency virus (FIV) is a retrovirus, similar to human immunodeficiency virus type 1 (HIV-1) in humans, that suppresses and inhibits activity of the immune system in cats. Studying FIV is important because humans and cats have similar immune responses to these respective viruses, suggesting that felines may be a plausible animal model for development of HIV-1 therapies. The Gag polyprotein features an N-terminal matrix (MA) domain that is responsible for assembly and targeting of Gag to the plasma membrane, a process that is vital for retroviral replication. Understanding the structure and function of FIV MA is necessary to characterize the assembly process and compare it to that of HIV-1 MA. Precipitation was observed during FIV MA purification, an observation that may be attributed to intermolecular interaction of the N-terminal myristate moiety of MA. We hypothesize that the myristate changes conformation, promoting precipitation at low pH. Expression and purification tests under variable pH conditions suggest that pH 8 promotes FIV MA solubility. Nuclear magnetic resonance spectroscopy will be applied to characterize structural changes associated with pH variation. This analysis will expand our knowledge on FIV MA structure and, ultimately, to the assembly process.

Introduction



HIV is a pandemic that infects more than 40 million people worldwide. To date there is no cure for HIV, and development of novel therapies has been largely impeded by absence of an appropriate animal model. Replication of the feline immunodeficiency virus (FIV) is similar to HIV, so cats may offer potential as an animal model for advancement of HIV treatment. Targeting of the Gag polyprotein (Gag) to the lipid bilayer is a process that is mediated by the N-terminal matrix protein (MA) which occurs in both HIV and FIV replication. The focus of this project is to characterize the function and structure of FIV MA.



FIV MA features a myristate moiety, a covalently linked, fourteen carbon saturated fatty acid, that plays a part in assembly. In order to characterize the structure and function of FIV MA, the protein must be isolated and purified. Despite successful FIV MA expression, conditions for purification have resulted in protein precipitation, thus reducing the protein yield. It is hypothesized that this aggregation is due to the myristate-myristate interactions on FIV MA. This work aims to identify favorable conditions for FIV MA purification.

Significance

- Felines have a similar immune response to FIV as humans do to HIV-1
- Studying the mechanism of assembly may lead to the discovery of novel drugs for HIV-1
- 2.5-4.4% of all household cats worldwide have FIV, so there is no need to infect healthy felines
- Cats are inexpensive to take care of and to obtain
- Understanding more about FIV may support the use of cats as an animal model for HIV

Methods

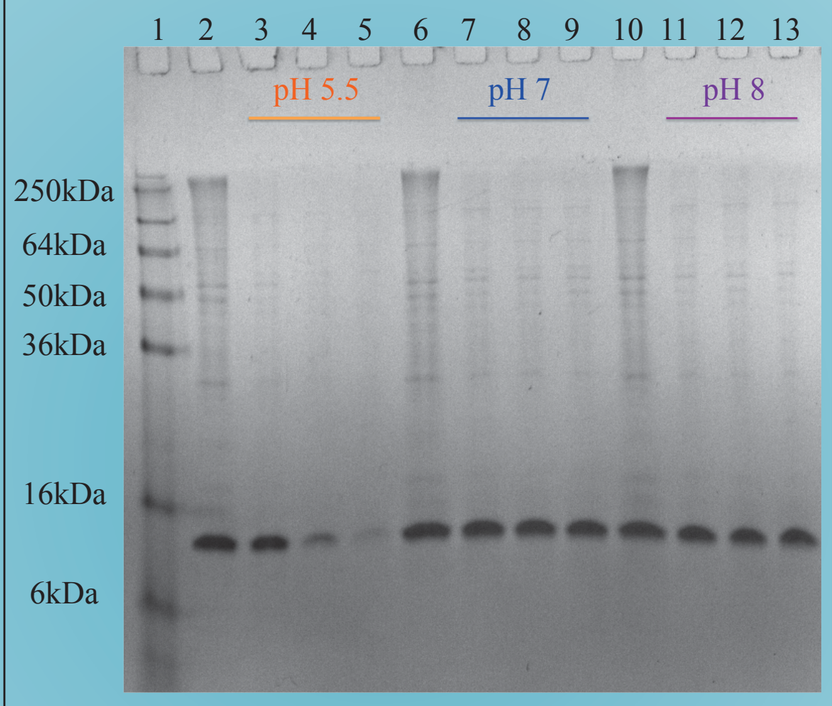
-Protein Expression
- Replicate vector in *E. coli*
- Supplement *E. coli* with myristic acid
- Induce with isopropyl-β-D-1-thiogalactopyranoside (IPTG)
- Cell Lysis

-Protein Purification (introduction of solubility issues)
- Affinity Chromatography
- Cation Exchange Chromatography

-Concentrate Protein
-Mass Spectrometry
-Nuclear Magnetic Resonance Spectroscopy

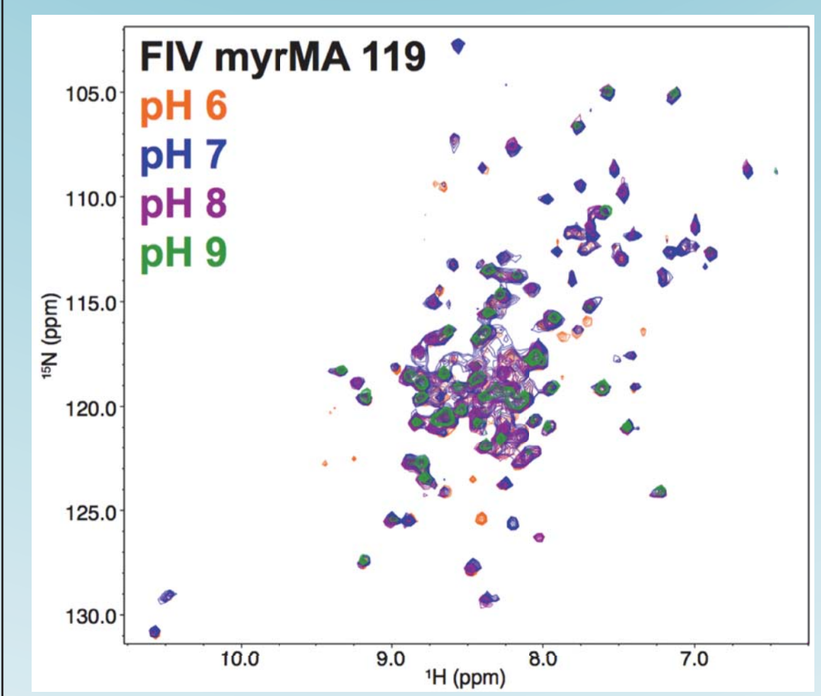
Results

FIV myrMA 119 solubility is effected by pH



1 - MW Marker	7 - post-dialysis
2, 6, and 10 - FIV myrMA 119 affinity chromatography eluate	8 - with 5µm filter
3 - post-dialysis	9 - with 0.45µm filter
4 - with 5µm filter	11 - post-dialysis
5 - with 0.45µm filter	12 - with 5µm filter

Heteronuclear Single Quantum Coherence (HSQC) of FIV myrMA 119 at pH 6, 7, 8, and 9



Discussion

This gel demonstrates that FIV MA (~14 kDa) solubility is effected by pH. Significant precipitate was observed in the pH 5.5 samples, so all samples in this analysis were filtered in order to detect soluble FIV MA. In pH 5.5 buffer, there is a low amount of protein retention in comparison to higher pH conditions of 7 and 8. After filtration, it can clearly be seen that at pH 5.5, FIV myrMA 119 is lost, illustrated by reduced intensity of the band at 14 kDa, meaning aggregation in aqueous solution occurred. Very little difference is observed in comparison of FIV MA in pH 7 and pH 8 buffer. It appears that pHs 7 and 8 are equivalent for protein solubility, however, over time, protein precipitation is observed in pH 7 conditions, perhaps due to eventual myristate exposure.

Heteronuclear single quantum coherence (HSQC) spectra were collected of FIV MA at varied pH in order to determine if structural changes were occurring. The signals in the HSQC are representative of the amino acids within the protein. The overlaid HSQC spectra demonstrate structural changes of FIV MA occur with varied pH. This result suggests that conformation change of FIV myrMA 119 may be occurring with varied pH.

Preliminary Conclusions

From the data collected, we can conclude that pH influences FIV myrMA 119 solubility and, potentially, conformation. At lower pH, solubility may be decreased due to a conformation change that exposes the hydrophobic myristate moiety linked to FIV myrMA 119. However, additional data is necessary in order to conclude that the poor solubility at lower pH is solely caused by the myristyl switch.

Future Directions

Collect additional NMR data to determine the placement of the myristate group when sequestered in FIV MA. This work will help to determine the role of the myristyl switch on FIV MA solubility, and further characterize the structure and function of FIV MA.

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References

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