Determining the 3D Structure of the Peach Latent Mosaic Viroid

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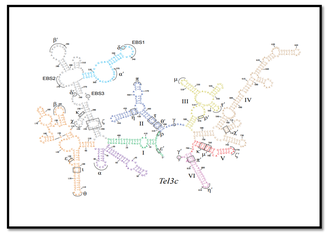
Introduction

The purpose of this study is to determine the 3D structure of the peach latent mosaic viroid. Viroids are very small, (246-401 nucleotides) circular RNA pathogens that infect plants and crops. Determining the 3D structure could offer insight into how the viroids infect their hosts, and how we could prevent infection or pathogenesis. The ultimate questions asked are "How does the 3D structure of the viroid affect its infectivity and pathogenesis?" and "How does the RNA recruit proteins within the plant to establish the infection?" We utilized gel electrophoresis, fast protein liquid chromatography (FPLC), and electron microscopy. To prepare samples for electron microscopy we attempted to flash freeze samples harvested from the FPLC to see if their structure would resemble that of the unfrozen samples. Tel-PLMVd ribonucleoprotein (RNP) is the attachment of the Tel protein to the Tel-PLMVd fusion RNA. The Tel and PLMVd RNAs have been combined to simplify the identification of PLMVd in imaging. In addition to electron microscopy we conducted an experiment in which we increased the amount of spermine in the Tel-PLMVd transcription to determine if it would inhibit the hammerhead ribozyme from self cleaving.

Background Information



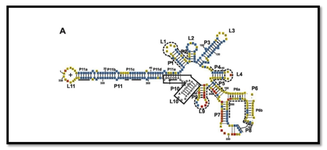


Figure 1 & 2:
Viroids are one of the smallest infectious pathogens known (between 246-401 nucleotides long). There are more than 30 known species that infect a large array of plants. They are single stranded circular RNA that do not encode for proteins. In a previous study conducted, the tertiary structure of the viroid was altered and it was seen that the viroid was no longer infectious[1].
Pictured on the left is the secondary structure of the Tel RNA (top) and the secondary structure of the PLMVd RNA (bottom)[2]. The two have been attached together in order to help determine the 3D structure of the PLMVd RNA.

Fast Protein Liquid Chromatography (FPLC)

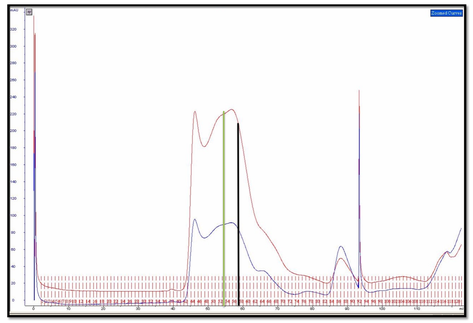


Figure 4:
The FPLC elution profile provides a graph of the UV absorbance of both protein (red) and nucleic acid (blue). The sample seen in Figure 3 eluted at 60 milliliters, therefore fractions 45-60 were analyzed further. Based off of our protein gel and RNA gel of these fractions, 55 (green line) and 58 (black line) were saved for imaging by electron microscopy.

Inhibition of Hammerhead Ribozyme

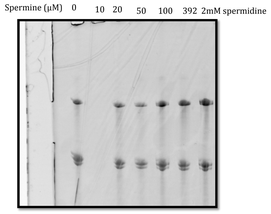


Figure 12:
A previous study showed that spermine could inhibit the self-cleaving of a different viroid [3]. To the left is the resulting gel when we tested different quantities of spermine and spermidine (0 or either, 10, 20, 50, 100, 392 spermine, and 2 mM spermidine.) We are able to see Tel PLMVd RNA (top band) as well as self cleavage products (bottom band).

RNA Gel

Figure 5:
We utilized gel electrophoresis to visualize the RNA in our FPLC fractions. In the RNA gel (left) we are able to see Tel-PLMVd lariat product, Tel-PLMVd RNA, and self cleavage products.

Protein Gel

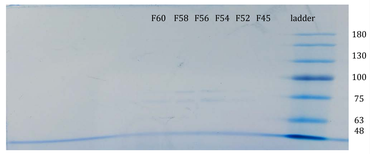


Figure 6:
Above is an SDS-Page gel (protein gel). As we can see, the bands are very faint. This is due to a low concentration of proteins. However we can see bands in F58, F56, F54, and F52 running between 63 and 70 kDa.

Conclusion & Future Studies

In conclusion we were able to determine that freezing the Tel-PLMVd RNP prior to electron microscopy will alter the visualization of the samples. Thus making it a not viable option for cryo-electron microscopy grid preparation. In addition we were able to see that the addition of spermine will not inhibit the self cleaving of hammerhead ribozyme within Tel-PLMVd.
The pursuit of the 3D structure will continue with the use of electron microscopy. In addition they will be testing the tertiary contacts in order to see the effects they have on the overall folding of the viroid. Finally they will determine the associated proteins necessary for the viroid to infect plants using a plant bioassay.

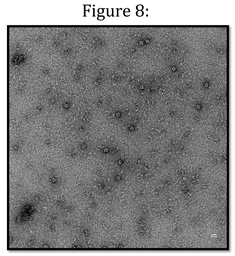
Methods & Materials

To prepare the Tel-PLMVd RNP for electron microscope grids, we used the FPLC to purify and isolate the correct complex. We later created both a SDS-PAGE gel to look at proteins and an acrylamide gel to look at RNA.

In addition to electron microscopy we evaluated whether or not spermine could prevent the self cleaving of the hammerhead ribozyme. We did this by adding different amounts of spermine to a mixture of Tel-PLMVd transcription which also included T7 buffer, 0.1 NTPs, 1% Triton x-100 and T7 polymerase.

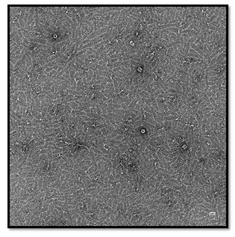
EM Images of Frozen Samples

Figure 8:



Figures 8 & 9:
These are images of fraction 55 (Figure 8) and 58 (Figure 9) which were flash frozen. The samples appear to be aggregated. We can see that the frozen particles do not remain in the same state as the non frozen samples.

Figure 9:



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Previous Experiment

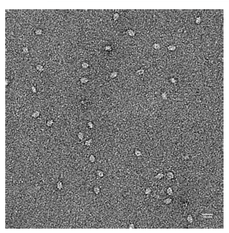
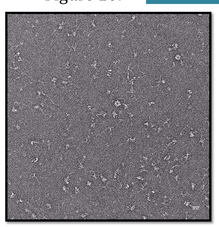


Figure 3:
The image on the left is a grid from a previous, successful experiment. Based off of this image our hopes were for the later images to resemble these particles. This is a clear image of the Tel-PLMVd RNP.

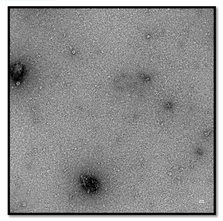
EM Images of Unfrozen Samples

Figure 10:



Figures 10 & 11:
These EM images are of fractions 55 (left) and 58 (right) which were never frozen. Due to aggregation these samples do not resemble previous images of Tel-PLMVd RNP seen in Figure 3.

Figure 11:



Literature Cited

1. Dube, A. *et al.* "The RNA strands of the plus or minus polarities of peach latent mosaic viroid fold into different structures." *RNA* 16 (2010), pp. 463-473.
2.Dubé, A., Bolduc, F., Bisaillon, M., & Perreault, J. (2011). Mapping studies of the Peach latent mosaic viroid reveal novel structural features. *Molecular Plant Pathology, 12*(7)
3. Kaddour, H., Vergne, J., Herve, G., & Maurel, M. (2014). Inhibition by polyamines of the hammerhead ribozyme from a Chrysanthemum chlorotic mottle viroid.*Biochimica Et Biophysica Acta (BBA) - General Subjects, 1840*(6)