2011

Methods of Protein Characterization

Michael Tieu
mtieu@pugetsound.edu

Methods of Protein Characterization

Michael Tieu*, Jeffrey S. Grinstead*

*Chemistry Department, University of Puget Sound, Tacoma, WA

Background

AppA is a protein in *Rhodobacter sphaeroides* that has been the topic of debate among scientists for the past several years with regards to the structure of the protein. It has been known that AppA has an effect on the activity of PpsR, which controls the gene expression of photosystems. There are two conflicting experimental structures (2IYG and 1YRX) of the protein, both of which claim to be taken in the dark phase (meaning when there is no light shining on the protein). The debate is about whether some slight differences in the structures represent the shift from the dark state to the light-induced state. The light-induced structure is known to have an exposed tryptophan (W104); therefore 2IYG may be the light-induced state because it has been found to have a tryptophan slightly exposed to the solvent (Dragnea et al).

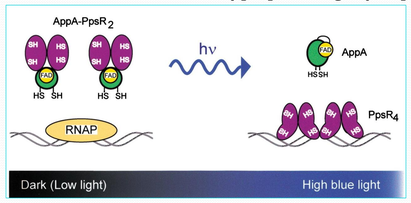
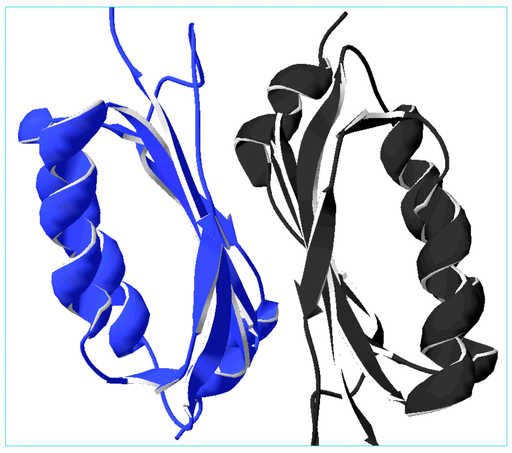


Figure 1. Mechanism of gene expression with AppA and PpsR

Pagano et al. found evidence that AppA exists as a dimer because the diffusion rates are different when it is measured in the light phase compared to the dark phase; the light phase had a slower diffusion rate than the light phase suggesting that it was a larger structure. They also performed an experiment where a paramagnetic relaxation enhancer was added to the solvent and some were shielded from being where it would normally go if it was a monomer, suggesting that there is a buried dimer. An experimental structure of the dimer was then constructed from the 2IYG structure along with data they gathered by using the protein docking program, HADDOCK. They were not able to construct a dimer from the 1YRX structure because the HADDOCK program did not give a unique dimer based on the 1YRX structure.

Structural Analysis of Two Crystal Structures of AppA

The purpose of this research experiment was to understand which differences between the two structures would explain why the 2IYG structure was able to converge to the dimer, while 1YRX was not able to.



-Pagano et al. constructed a light-induced dimer structure using 2IYG as the monomers; 1YRX did not give a dimer in Pagano's study.
-The side view (left) shows how the dimer is made up of two 2IYG structures bonded together at their beta strand sheets.
-The frontal view (right) shows how the 2IYG structures stack onto each other to form the dimer.
-Interactions (Hydrogen bonds and London dispersion forces) between the two beta sheets were analyzed and recorded.

Figure 2. Theoretical dimer structure of the AppA protein made from the 2IYG structure.

Sidechains at the Dimer Interface

-The shift of Gln107 and Ser109 could suggest that 1YRX does not form a dimer because those amino acids in the 2IYG dimer interface form strong hydrogen bonds and some London dispersion forces. The same interactions would not form for 1YRX because Gln107 and Ser109 are shifted farther down along the beta strand compared to 2IYG.

-The hydrophobic amino acid sidechains in the beta sheet for 2IYG are pointed in different directions compared to 1YRX (Phe55, Trp64, Phe62, Arg100), which changes the shape of the surface.

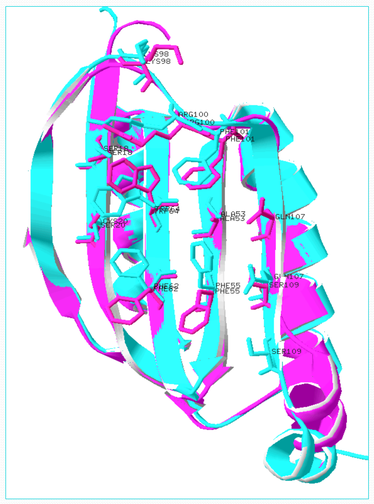


Figure 3. Overlay of the 2IYG and 1YRX structures with highlighted amino acid sidechains found to have interactions in the dimer structure.

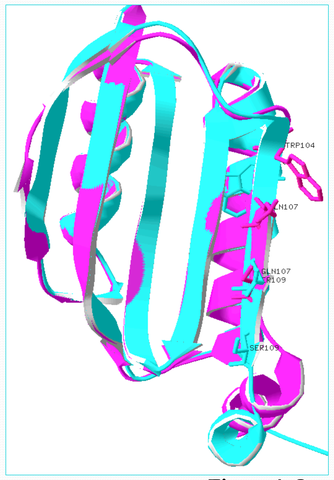
Acknowledgements

I would like to the University of Puget Sound and the chemistry department for the wonderful education they have provided, as well as the opportunity to conduct research at the amazing institution. I would also like to take the time to thank Jeff Grinstead for guidance, patience, and much needed humor during times of struggle.

References

Dragnea, V., Arunumar, A. I., Yuan, H., Giedroc, D. P., and Bauer, C. E. (2009) Spectroscopic studies of the AppA BLUF domain from *Rhodobacter sphaeroides*: addressing movement of the tryptophan 104 in the signaling state. *Biochemistry 48*, 9969-9979.

Pagano, K., Grinstead, J.S., Madl, T., Kaptein, R., and Boelens, R. (2011), "From sparse NMR data to structural insights into the light-induced dimer of the AppA BLUF domain." manuscript in preparation.



Dimer Interface Differences

-Gln107 and Ser109 are shifted farther down in the 1YRX (blue) structure compared to the 2IYG (purple) structure.
-The sidechain of Trp104 is pointed outwards in the 2IYG (purple) structure, while 1YRX (blue) has the Trp104 pointing inwards.
-The side view (right) shows that the 2IYG structure has a longer loop before the fifth beta strand, which explains how the two structures differ in the placement of Gln107 and Ser109.
-Dragnea et al. saw differences in the two experiments with regards to solution conditions and C terminus lengths (1YRX was AppA17-133, 2IYG was AppA1-126), which could explain part of the differences between the two structures of AppA.

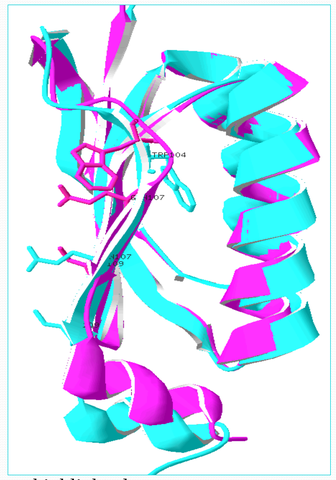


Figure 4. Overlay of the 2IYG and 1YRX structures with amino acids in different positions highlighted.

Conclusion

Many studies have focused on the tryptophan being the main functional difference because of its characteristics of being exposed to the solvent or buried and interacting with conserved Gln63 near the flavin chromophore, but our study of the dimer shows that it does not have any interactions with respect to the dimer interface. Instead, we have found that shifted amino acids, such as Gln107 or Ser109, should be given more focus because they are involved more in the dimer interface and are very different from each other in the conflicting structures of 2IYG and 1YRX.

Mass Spectrometry

Background

Liquid chromatography-mass spectrometry (LC-MS or HPLC-MS) is an analytical chemistry technique that is extremely useful because it involves physical separation along with mass analysis of samples at a high level of sensitivity and selectivity. The most common uses of LC-MS include the analysis of chemical compounds, protein characterization, and various areas in drug development/research. The HPLC-MS machine at UPS has been notorious for having issues working in the past, but it seems that most of the major issues have been addressed. On that note, proper procedures for using the instrument safely and accurately are needed in order to prevent more damage in the future.

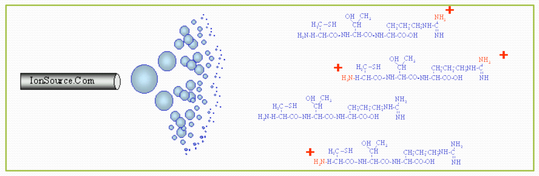


Figure 5. Ionization of samples in the LC-MS

Protocol for the LC-MS

The purpose of this research experiment was to implement a MS method to measure the mass of proteins and to identify an approximate limit of detection.

-The sensitivity of the instrument was tested with various samples of lysozyme at different concentrations and sample sizes.

-Spectra of other proteins such as P450 eryF or MalA did not give interpretable mass spectra (Figure 7, 8).

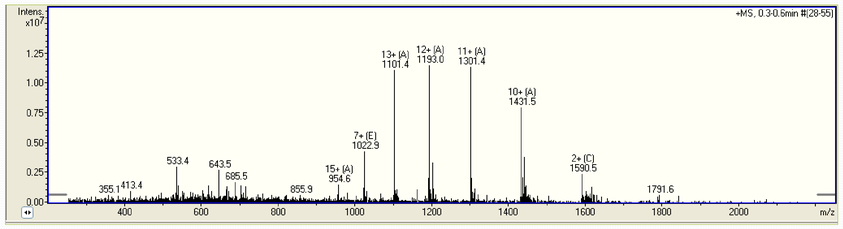


Figure 6. Mass spectrum of 1 μM lysozyme with a 5 μL injection

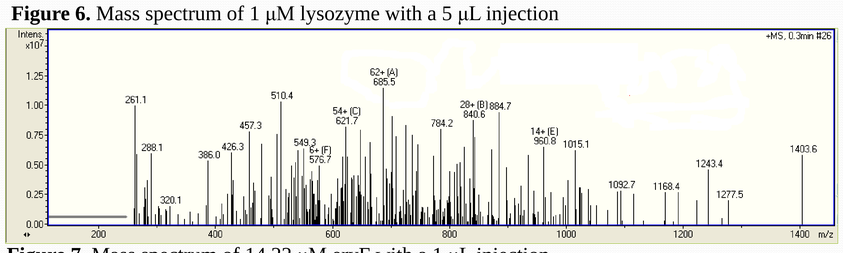


Figure 7. Mass spectrum of 14.22 μM eryF with a 1 μL injection

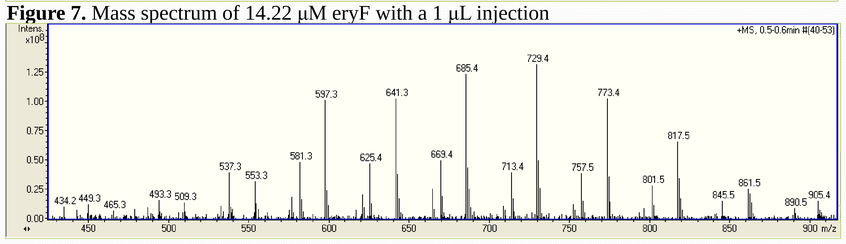


Figure 8. Mass spectrum of unknown malA sample with a 0.1 μL injection

Conclusion

This experiment demonstrated the possible errors that could occur if samples are not prepared properly before being analyzed with the LC-MS, but it also showed how accurate the instrument can be as seen from the lysozyme experiments if the correct precautions are taken beforehand.