Formulations for Success

Alfredo Perez, Biochemistry major at Southwestern College alfredoperez.ap@gmail.com

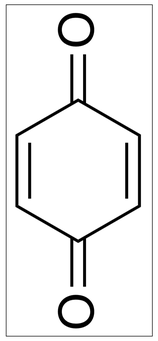
Christina Newell, Biochemistry major at San Diego State University x-tinanewell@hotmail.com

Ryan Hagar, Kinesiology/Biology major at Southwestern College rhagar05@yahoo.com

SWC: NSF Grant # DUE0653291
SDSU: NSF Grant # DUE0653234

Introduction

Most of the protein found in a given cell is multimeric. This is due to a variety of weak interactions that are spread over a large area in the interfaces between the individual protein monomers. In order to evade the challenges of controlling these weak forces, we use metal-ligand interactions to guide protein self-assembly. Using site-directed mutagenesis, cytochrome cb_{562} was modified so that it can coordinate to metals at specific locations on the protein to form tetramers. The structure was verified with x-ray diffraction. This tetrameric construct is known as Metal Binding Protein Construct-1 (MBPC-1). Further modifications gave rise to disulfide bridges and hydrophobic interactions between the individual monomers, which stabilized the multi-protein complex even in the absence of the coordinating metal. This newly designed construct is known as Rosetta Interface Designed Complex-1 (RIDC-1). We are currently trying to get a stable Cu(I)-bound structure. Once this is done, we should be able to make further modifications to cyt cb_{562} in order to cause reduction-oxidation activity within the coordination sites.



Structure of quinone.

The Smith lab is working on developing and understanding the mechanism for how electron-proton transfer and hydrogen bonding work together in redox reaction couples. Since the major energy transduction processes in living organisms, such as the production of mitochondrial ATP and photosynthesis, are centered around these reactions, it is vital to understand these mechanisms. Quinones, organic compounds derived from aromatic compounds, are prime examples of redox systems. These compounds can be studied using electrochemistry.

There exists drugs in current use that reduce pain and inflammation that cause negative side affects. Systemic exposure to the active drug, which is used by oral medications has been proven to be a major cause of this. An alternative is the transdermal drug delivery platform, which utilizes transdermal carriers, to provide site specific treatments, which has been proven to reduce systemic exposure to the active drug and therefore reduces negative side affects. This research studies the retantion of diclofenac sodium, a NSAID, in shed snake skin as the model membrane.

Materials & Methods

Different versions of protein are designed using computational docking programs. These alterations are carried out through site-directed mutagenesis. The protein is then expressed with BL21 (DE3) cells, which are chemically competent *E.coli* cells that are engineered to over-express protein. The protein is isolated with column chromatography. Once the protein is isolated, it is mixed with a metal ion solution, along with a variety of buffers, precipitating agents, and salts in order to allow for the coordination of the protein with the metal ions. When crystals form , X-ray diffraction is used to determine the chemical structure of the multi-protein complexes. Sedimentation velocity experiments are used to determine the oligomerization state of the multi-protein complexes in solution.

Alfredo using a micropippettor.

Cyclic voltammetry (CV) is used to study the electrochemical properties of an analyte. Voltammograms are run using a potentiostat hooked up to a PC equipped with software that can be used to record and analyze data.

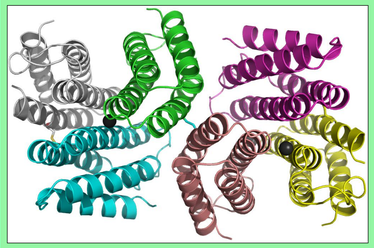
Potentiostat used in Christina's lab.

Christina extracts methanol to react with Duraquinone.

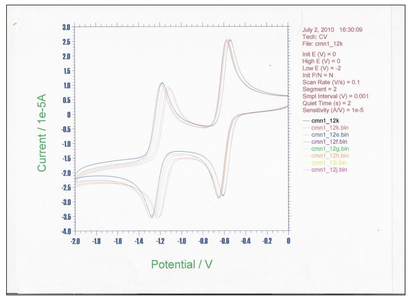
Shed snake skin and 4 dram control vials were placed in a 32°C oven overnight for 24 hours and weighed. This was repeated until the mass reached equilibrium. Then 50µl of formula was applied to each respective snake skin and vial, and exposed to room temperature for 24 hours and weighed. This was repeated until the mass reached equilibrium. Formulas that were retained in the snake skins were extracted by soaking the snake skins in ethanol for 24 hours in darkness. The extracted formulas were then run through a HPLC to analyze the amount of diclofenac that was retained In the snake skin.

Ryan applying formulas to snake skin.

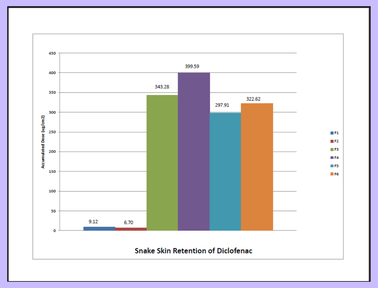
Results



Hexamer crystal structure of $^{C96}{}^{M74}$RIDC-1 with Cu(I). Each disulfide linked monomer (cytochrome cb_{562}) is shown in a different color. The Cu(I) ions are represented as black spheres.



Cyclic Voltammogram of Duraquinone with methanol.



Retention graph showing the amount of Diclofenac retained in snake skin.

Conclusion

We still do not know which amino acids on the protein coordinate to Cu(I). According to the crystal structure, we were expecting a hexameric form to be present in solution. However, sedimentation velocity experiments indicate that the structure is tetrameric. ^{C96}RIDC-1 favors dimers. We think that the M74 mutation is influencing the coordination to the metal, but we need crystals with better resolution to determine the amino acids that are bound to the metal. Currently, we are also trying out different versions of protein mutants and determining the amino acids coordinated in the resultant oligomers.

Several CV's were run on four different quinones in 1mM concentrations and all four showed two peaks. However, the second peak was smaller than the first. The difference in the peaks was less when a bulkier quinone was used. In theory, both peaks should be equal; which strikes up the hypothesis that the radical cation is forming dimers on the surface of the electrode. When a guest was added to the solution, the peaks shifted to a more positive potential. This means that the quinone had an easier time accepting the electron. The data for these experiments on quinones will be used in comparison with data obtained from experiments involving phenylenediamines, which are hypothesized to undergo similar mechanistic pathways.

The experiments performed by Christina Newell not only made a contribution to the overall project but also allowed her to learn valuable techniques and procedures that cannot be obtained from a textbook.

It is unclear if evaporation plays a factor in Diclofenac retention. Formulas 3, 4, 5 and 6 have shown that complex formulas are needed for greater retention of diclofenac versus the simple Formulas 1 and 2. However, more experiments must be performed on new formulations to discover optimal retention and evaporation rates for any given situation.

Acknowledgements

- We would like to thank Annette Medina-Morales, Dr. Akif Tezcan, Dr. Diane Smith, Laurie A. Clare, Dr. Nadir Buyuktimkin, and Dr. Servet Buyuktimkin for their mentorship.
- Thank you to the MESA Alliance Program, LIPP Family Foundation, National Science Foundation, and the Howard Hughes Medical Institute for providing opportunities to the internship experience.
- Last and final thanks to our family, friends, and those who support throughout the internships.