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Effects of Portal Protein Primary Structure Mutations on Viral Genomic Packaging Capabilities

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Abstract

Bacteriophages are bacterial viruses that make up the most numerous and varied “species” in the world, outnumbering bacteria by a factor of ten. Despite this fact, all types of bacteriophages are composed of the same fundamental structure. As such, you can look at one bacteriophage and have a good understanding of another’s function and structure. So our lab uses bacteriophage phi29 as a model for extrapolation to other bacteriophages.

Introduction

All bacteriophages, not just phi29, have portal proteins. Proteins are large molecules that are composed of amino acids. Each of the amino acids that compose proteins have a unique set of properties and altering these properties (through an alteration of amino acid sequence) can greatly alter protein function. While we could focus on altering a plethora of properties, we decided to focus primarily on the charges of amino acid side chains. The wild type phage is so effective at packaging DNA to the gradient build-up, which is aided by the charges of amino acid residues in the protein’s channel. So mutant proteins were harvested and used for analysis.

Reagents

In this experiment, there are relatively few reagents needed the most important of which are proteoliposome solutions prepared by our lab(3), a buffer solution made to mimic in-vivo ion conditions, and the protein solutions. The mutant protein solutions used included E197K, E197Q, E197K D202K, in addition to the wild type protein.

Experimental

A synthetic bilayer was established in a two chamber system. This two chamber system was used to re-enact in-vivo situations in-vitro by allowing us to alter buffer conditions (salt used, pH) while also allowing us to measure current at different applied voltages. These different voltages played the part of the membrane potential.

Once the synthetic bilayer is appropriately established, a small sample of protein is placed onto the membrane and the electric properties were measured and recorded. Using ohm’s law as a benchmark, we can relate resistance and voltage to changes in current.

Results and Discussion

We can see that the mutations of amino acid residues that influence the charge of the inner channel do influence a plethora of portal protein properties. The exact relationship to the actual genomic packaging are still unknown due to limited resources but this is promising for potential applications in medicines and biophysics.

Acknowledgments

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Sources

Data Collection and Analysis

Data was collected via single molecule analysis using a synthetic lipid bilayer through the patch clamp method. This yielded a current-voltage graph which allowed us to know when one portal protein had inserted itself in the bilayer.

These data were then used to calculate the ion selectivity of the portal protein through a derivation of the Nernst Equation.

\[ E_m = (2.303RT/F)(1 - \exp(-\frac{zFV}{RT})) \]

(1)