Importance of Isoleucine 55 for Rotor-Stator Interactions in E. coli F1FO ATP Synthase

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Abstract

ATP synthase, a ubiquitous biological nanomachine, is responsible for synthesizing the majority of adenosine triphosphate (ATP) in cells. The process of synthesizing ATP uses a unique rotary mechanism, which involves two motors, F1 and FO where protons are translocated in FO. Cryo-electron microscopy (cryo-EM) maps have given insight into the structure of Escherichia coli (E. coli) ATP synthase; however, they do not explain the intricacies of how protons drive rotation. Previous studies showed that proton translocation occurs at the subunit a/c interface (located in FO) and that some amino acid residues are important for function; among these is isoleucine 55 of subunit c (cI55). We are trying to elucidate what chemical properties are essential for functionality at position 55, which is located on the second transmembrane helix (TMH2) of subunit c. Changes in the side chain will be imposed using site directed mutagenesis via polymerase chain reactions (PCR) and chemical modifications via methanethiosulfonate reagents and functionality observed using fluorescence spectrometry. So far, replacing isoleucine with alanine (cI55A) resulted in H+ pumping that behaves similarly to that of the wild type. This result leads us to believe that steric bulk is not an essential property at this position, and we are currently looking at the importance of hydrophobicity.

Background

All cells require energy to survive. Cells engage in a repetitious cycle in which they convert nutrients that they obtain from their environment into energy via biochemical processes. The energy that cells extract from food subsequently gets stored into a proton gradient in the mitochondria. The mitochondria, also known as the powerhouse of the cell, is the site where adenosine triphosphate (ATP) is synthesized which is driven by differences in the proton gradient. ATP synthase is a ubiquitous biological nanomachine responsible for synthesizing the majority of ATP in cells. ATP synthase is composed of two motors, F1 and membrane embedded FO. ATP is synthesized in F1 by inducing conformational changes which are driven by using a unique rotary mechanism that takes advantage of the proton gradient across the cell membrane in FO.1,2 Due to the lack of high resolution structural data, the intricacies of the FO mechanism are not known.

FO is composed of multiple subunits of which a and c are of the most importance. These subunits work together to generate rotation using the proton gradient across the membrane. Researchers suggest that the interactions between subunit a and c are integral to the mechanism that drives the rotation of FO. Subunit a, the stator, is where protons enter FO using an entry half-channel. A conserved arginine residue which is positively charged lies in subunit a causing protons then to move to subunit c, the rotor. Due to the discontinuous channel, protons are forced to rotate about the c-ring before they can exit using the exit half-channel located at the a/c interface.3 Previous studies have substituted cysteines for the amino acid residues on subunit c which resulted in identifying specific amino acids that are important to the mechanism that drives ATP. Isoleucine at position 55 (cI55) was identified as one of those important residues.4 In the Steed research group, we are determining which chemical properties are essential for the functionality of FO in both the ATP synthesis and hydrolysis directions. The results from this research can then be extrapolated towards the advancement of medical research regarding how antibiotics inhibit at the a/c interface against mycobacteria e.g.: Bedaquiline which is used to treat multidrug resistant

tuberculosis (MDR-TB).5

Methods

This study will utilize site-directed mutagenesis via polymerase chain reactions (PCR) and chemical modifications via methanethiosulfonate (MTS) reagents. PCR will be performed using oligonucleotides with the desired amino acid substitutions, isoleucine at position 55 of subunit c to serine (cI55S), threonine (cI55T), and tyrosine (cI55Y) respectively. Genetically modified mutants will be sequenced to confirm that the desired alterations were made successfully. Chemical modifications will be introduced to a cysteine substituted amino acid side chain by using various MTS reagents, which will test for the importance of hydrophobicity. The purpose behind modifying a cysteine substituted amino acid side chain is due to its special reactivity. The chemical structure of cysteine allows for disulfide bonds to be made when reacted with the MTS reagents thus varying the size of the side chain. The chosen MTS reagents, methyl MTS, ethyl MTS, propyl MTS, butyl MTS, phenyl MTS, and benzyl MTS are a series of substituents that increase in hydrophobic bulk. Hydrophilic bulk will also be tested using MTS reagents, which will result in the additions of hydroxyethyl, carboxymethyl, carboxyethyl, and carboxypropyl substituents respectively. All chemical modifications will be tested for reactivity using an Ellman's reagent assay. Then the successfully prepared mutants of the F1FO ATP synthase will be tested for functionality in both the ATP synthesis direction (Luciferin/Luciferase-based ATP synthesis activity assay) and in the hydrolysis direction (ATP-driven H+ pumping assay) using fluorescence spectroscopy to determine which chemical properties are essential at the a/c interface.

Budget and Justification

10 weeks full-time student stipend (5/29-8/3) $1500
MTS Reagents for chemical modifications from Toronto Research Chemicals (TRC)

Catalog #	Reagent Name	Cost
M321500	Methyl MTS	$45/100 mg
E925010	Ethyl MTS	$95/50 mg
P838250	Propyl MTS	$95/50 mg
B693275	Butyl MTS	$90/50 mg
P335675	Phenyl MTS	$95/5 mg
B285500	Benzyl MTS	$105/50 mg
H942250	2-Hydroxyethyl MTS	$95/5 mg
C180500	Carboxymethyl MTS	$140/10 mg
C178100	2-Carboxyethyl MTS	$75/50 mg
C181240	3-Carboxypropyl MTS	$95/5 mg

Total $930 Stipend + Materials Total $2,430

The MTS reagents requested will allow for the comparison of nonpolar versus polar group interactions' effect on ATP synthase functionality. The results from these studies will yield insight behind the chemical properties that are essential for the mechanism that drives rotation of F1FO ATP synthase.

Timeline

May 29, 2018-June 29, 2018 Generation of new mutants via PCR reactions using cI55S, cI55T, and cI55Y oligonucleotides and send for sequencing.
June 29, 2018-July 20, 2018 Chemically modify cI55C mutant with MTS reagents to observe what effects nonpolar versus polar groups have on functionality.

July 20, 2018-Ausugts 3, 2018 Test all mutants using fluorescence spectroscopy.

Publication Outlet

The results from this research will be submitted to UNC-Asheville Journal as well as to a journal specific to the biochemistry discipline such as, The Journal of Biological Chemistry (JBC) and presented at the UNCA Undergraduate Research Symposium as an oral presentation.

References

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(3) Fillingame, R. H.; Steed, P. R. Half Channels Mediating H+ Transport and the Mechanism of Gating in the Fo Sector of Escherichia Coli F1Fo ATP Synthase. Biochim. Biophys. Acta - Bioenerg. 2014, 1837 (7), 1063–1068.
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(5) Preiss, L.; Langer, J. D.; Yildiz, Ö.; Eckhardt-Strelau, L.; Guillemont, J. E. G.; Koul, A.; Meier, T. Structure of the Mycobacterial ATP Synthase Fo Rotor Ring in Complex with the Anti-TB Drug Bedaquiline. Sci. Adv. 2015, 1 (4), e1500106.