Determining the Mechanical Properties of the *E. coli* Methionine Transporter

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Abstract

Adenosine Triphosphate Binding Cassette (ABC) transporters constitute a superfamily of active transporters embedded in the cellular membrane. They consist of two highly conserved nucleotide-binding subunits which bind and hydrolyze ATP, and two diverse transmembrane subunits which provide a pathway for the substrate to pass through the membrane. ABC transporters serve a broad range of vital functions. Various conditions like cystic fibrosis and Stargardt disease are caused by defunct ABC transporters, and certain medical complications like antibiotic drug resistance are linked to promiscuous ABC transporters. Despite the importance of these transporters in crucial biological processes, the mechanisms of many transporters are yet to be solved. While many universal features of ABC transporters have been identified, the step-by-step process by which individual transporters move the substrate are a mystery.

To further understand the mechanism of ABC transporters, we are studying the E. coli methionine ABC importer MetNI. Because the bacterium needs to vary methionine import based on cellular needs, MetNI ATPase activity and coupled substrate transport must be properly regulated. Our current goal is to understand the mechanism of MetNI ATP binding and hydrolysis using a real-time ATPase assay. Here we present our preliminary work on analyzing the kinetics of MetNI ATP usage under varying conditions and with different mutations. This detailed study of MetNI kinetics will ultimately provide insight into the mechanism of methionine import, which may be more broadly applicable to the ABC transporter superfamily.

Introduction

MetNI belongs to a superfamily of active transporters known as ATP Binding Casette (ABC) transporters. ABC transporters harness the energy of ATP in order to translocate molecules across the cellular membrane against the concentration gradient. MetNI is an *E. coli* methionine importer consisting of two homodimers with each dimer pair making up the transmembrane domain (TMD) and nucleotide-binding domain (NBD) respectively (1).

MetNI is additionally paired with a substrate-binding protein (SBP) called MetQ which exists in the bacterium's periplasm and increases the transporter's affinity for the substrate. It is believed that MetNI favors the canonical model of transport for methionine in which the SBP binds to and delivers the substrate to the transporter. In contrast, it is believed MetNI favors the non-canonical model for methionine derivatives in which MetQ binds to the transporter, forming a high-affinity complex to facilitate substrate transport (2).

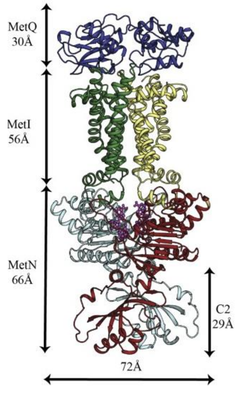

Fig 1. Crystal structure of MetNI complexed with MetQ (2).

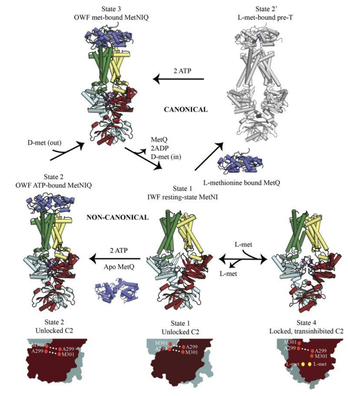

Fig 2. Canonical and non-canonical models of the MetNI transport mechanism (2).

Objective

Our goal is to understand the mechanism of the methionine transporter MetNI. We will accomplish this by:

- Purifying MetNI proteins and MetNI mutants, including chimera proteins
- Optimizing and running ATPase assays

Methods

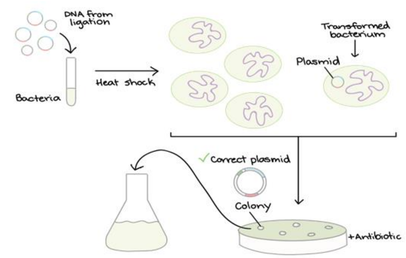

Fig 3. Transformation and expression of MetNI. Cells are heat-shocked to allow plasmid entry and selected for antibiotic resistance using ampicillin and kanamycin depending on the plasmid or plasmids (3).

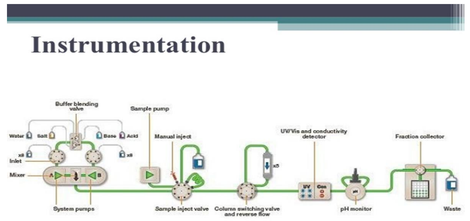

Fig. 4. Instrumentation of fast-protein liquid chromatography (4).

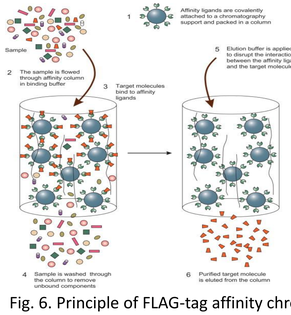

Fig. 6. Principle of FLAG-tag affinity chromatography for MetNI chimera purification. Proteins containing the FLAG tag bind to the FLAG antibodies attached to the resin, and the attached proteins are eluted out via competition with FLAG peptide (6).

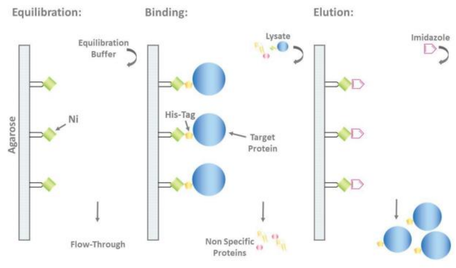

Fig. 5. His-tag affinity chromatography for MetNI purification. Proteins containing the six-histidine tag bind to the nickel on to the resin, and the attached proteins are eluted out via competition with imidazole (5).

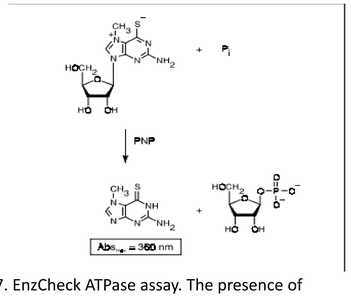

Fig. 7. EnzCheck ATPase assay. The presence of hydrolyzed phosphate allows PNP to hydrolyze MESG, removing the nitrogenous base which can be detected at 360 nm (7).

Results

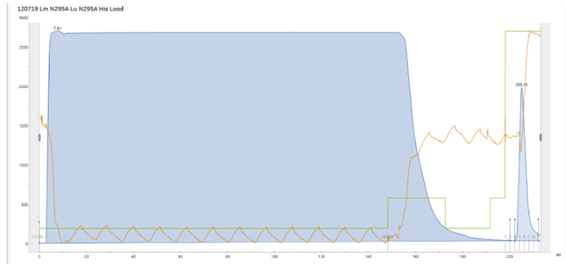

Fig 8. Chromatogram of MetNI N295A/E166Q His-tag purification. The peak at 203mL is the eluted protein of interest; the broad signal prior is eluted protein during the protein loading process.

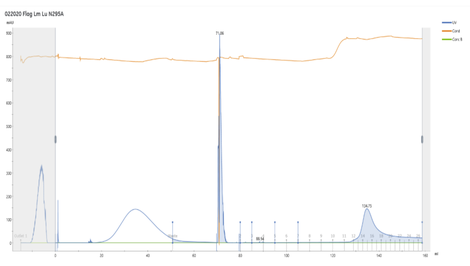

Fig 9. Chromatogram of MetNI N295A/E166Q FLAG-tag purification. The peak at 134mL is the eluted protein of interest; the peak at 40mL is eluted His-tag protein during the protein loading process; and the peak at 71mL is air during the FLAG peptide loading process.

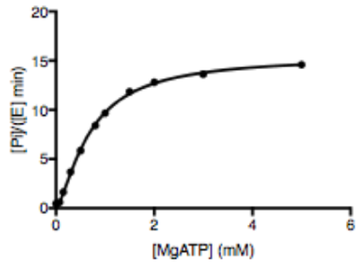

Fig. 10. Michaelis-Menten curve of MetNI N295A/E166Q ATPase activity.

Vmax (min^{-1})	h	Km (mM)
15.46	1.229	0.7463

Table 1. ATPase assay kinetics data for the MetNI N295A/E166Q chimera.

Progress and Future Work

- Successfully purified MetNI wild type (WT), mutants, and an N295A/E166Q chimera
- Successfully optimized the ATPase assay
- We will purify different chimeras and run ATPase assays on said chimeras
- We will purify various forms of MetNI – including chimeras – in nanodiscs and run ATPase assays
- We will run ATPase assays with MetQ

References

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