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Substrate Specificity in ABC Transporters Using the E. coli Methionine Import System

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Abstract

ATP-binding cassette (ABC) transporters use the **energy of ATP** to move substrates across membranes **against a concentration gradient**. The role of ABC transporters is crucial in several essential cellular functions. Mutations in human ABC transporters have been linked to several conditions, including **cystic fibrosis, liver disease, and diabetes**. Despite their central roles in homeostasis, **the mechanism of ABC transporters remains poorly understood**. Our research is focused on studying an ABC importer in *E. coli*, as a model system, to examine the **mechanism of substrate specificity and transport**. The bacterial methionine import system consists of a membrane-embedded transporter, **MetNI**, and a cognate binding protein, **MetQ**. Studies have been done of MetQ substrate specificity by purifying several variants of MetQ. **Characterized by the binding affinities** for methionine derivatives via the **Isothermal Titration Calorimeter (ITC)**. Our data confirms that these mutations affect the binding affinities for methionine derivatives. For some mutations it removes the ability to bind to methionine itself. With these binding affinities in hand, further, experiments with the membrane-embedded transporter, MetNI, will be done to dissect the mechanism of ABC transporters.

Objective

Our research is focused on studying a bacterial ABC model importer in *E. coli,* to better understand the mechanisms of substrate specificity and transport. To understand the substrate specificity, we are examining the substrate binding protein (SBP) MetQ, by mutating single amino acid residues in the binding pocket and measured how these mutations affect the binding of methionine isomers.

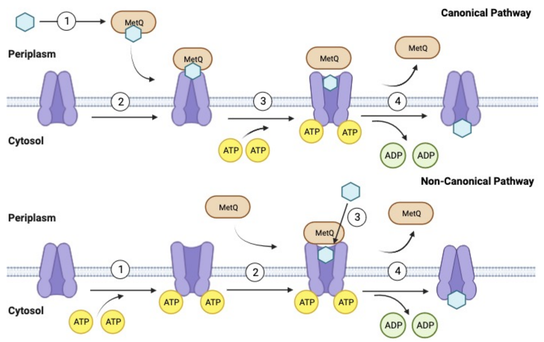

Figure 1. Two Models for ABC Import Systems Cartoon representation of ABC import systems. The purple membrane protein represents MetNI, and the blue hexagon is substrate, methionine. (*Top*) In the canonical model, substrate is sequestered by MetQ and delivered to MetNI. (*Bottom*) The non-canonical model, substrate passes through a pre-formed MetNI-Q complex.

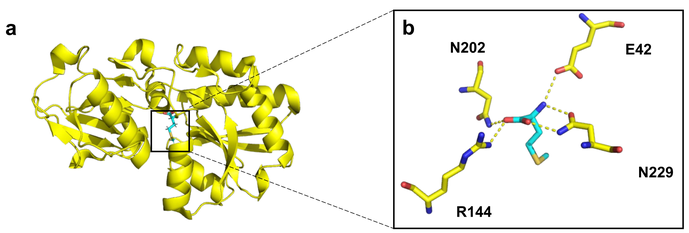

Figure 2. ABC Importer Models (a) Crystal structure of *E. coli* L-methionine bound MetQ. (b) Hydrogen bonds between L-Met and key residues shown in yellow dashes. PDB 4YAH.

MetQ Expression & Purification

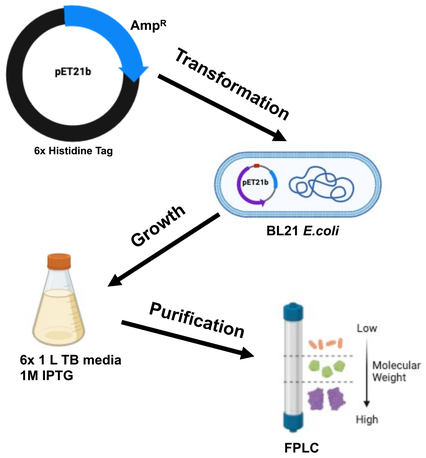

Figure 3. MetQ Growth & Purification. Cartoon representation of the purification process MetQ. First part is a transformation of the Lf-MetQ plasmin into a BL21 *E.coli* competent cells. After the transformation cells were grown in 1 L TB media with 100 mg/mL ampicillin reaching OD ~ 2. Then inoculated with 1M IPTG. Final step in multistep purification, initially using a Ni^{2+} affinity column. Bound proteins are eluted out with Buffer B (25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 400 mM imidazole, 5 mM BME). Eluted protein is put in a desalt column to change buffers from Buffer B to Buffer A (25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM BME). Eluted protein from desalt column will be injected into a size-exclusion column with Buffer A. Lf-MetQ was a gift from Douglas Rees.

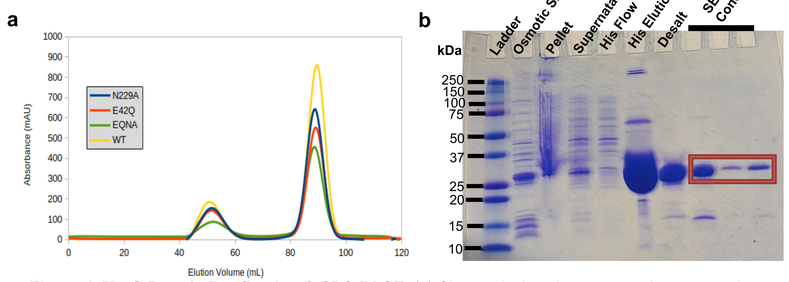

Figure 4. MetQ Protein Purification & SDS-PAGE. (a) Size-exclusion chromatography was used to separate aggregated protein (eluting at 50 mL) from monomeric MetQ protein (eluting at 90 mL). Importantly, the wild-type and mutant versions of MetQ eluted at the same volume (b) Each lane corresponds to a different step in the purification of MetQ from *E.coli.* The red box highlights purified MetQ. The band is slightly above the 25 kDa standard, which corresponds to the published value for MetQ of 30 kDa.

Isothermal Titration Calorimetry (ITC)

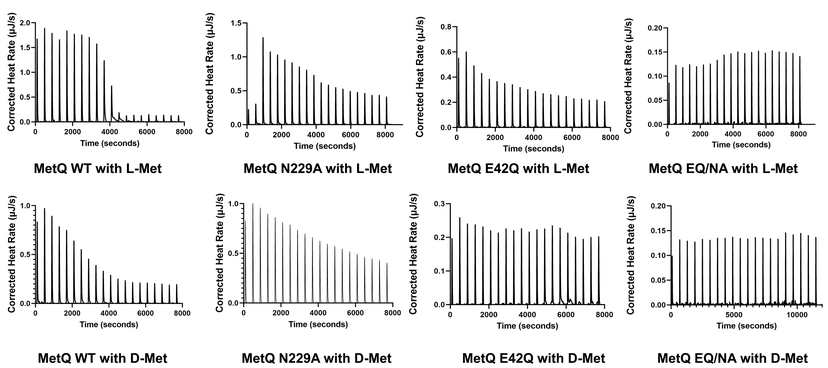

Figure 5. Representative thermograms of methionine binding to MetQ variants. All MetQ variants were dialyzed overnight at 4℃ in buffer containing 25 mM Tris pH 7.5 and 150 mM NaCl using a 10-kDa molecular mass cutoff Slide-a-Lyzer MINI dialysis device (Thermo Scientific). The next day, samples were recovered and cleared at 90,000 rpm for 20 min in a TLA100 rotor. L- and D- methionine solutions were prepared in the buffer used for dialysis. Binding data were collected on a TA instruments Nano ITC calorimeter. For wild-type protein, 50 µM MetQ was titrated with 500 µM methionine. For MetQ mutants, 100 µM protein was titrated with 500 µM methionine. For all experiments, 2.5 µL of titrant was injected into the sample cell 20 times with 400 seconds between injections.

Results

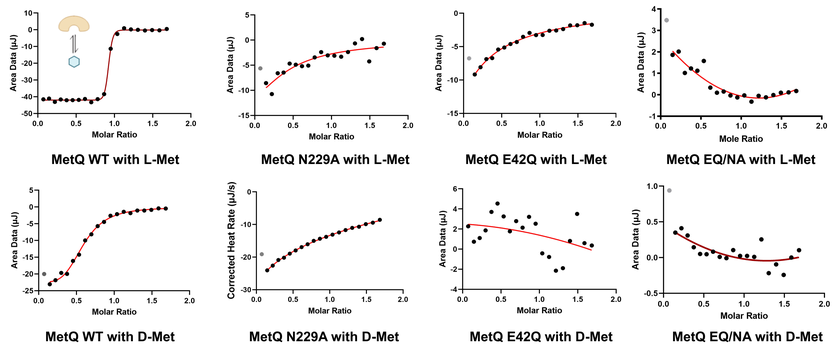

Figure 6. Binding curves of D- and L-methionine to MetQ variants. Data were fit to a one-site model using Prism software.

MetQ variant	L-Met (µM)	D-Met (µM)	c-values
wild-type	0.019 ± 0.014	5.3 ± 2.2	L- [>1000], D-[7 - 31]
N229A	0.46 ± 0.45	180 ± 170	L- [3 - 700], D-[0.3 - 1.1]
E42Q	68 ± 41	--	L- [0.2 - 2]
N229A/E42Q	--	--	--

Table 1. Summary of dissociation constants derived from ITC titrations. Binding was not observed for titration of E42Q with D-Met and for the titration of the double mutant NA/EQ with either D- or L-Met. All measurements were done in triplicate using at least two different preparations of each MetQ variant. Error values represent the standard deviation. C-values were calculated based on the stoichiometry (n) by the molar concentration in the cell ($[M]_{cell}$), divided by the dissociation constant (K_D).

Conclusions & Future Research

Our data show that **mutated amino acid resides** in the MetQ binding site **reduce the affinity** for L-methionine by approximately **100-fold**. Our preliminary results show that **wild-type MetQ** can **bind to both L- or D-methionine**, while a glutamate to glutamine (**E42Q) mutation loses the ability to bind to D-methionine** but retains the ability to bind the L-isomer.

Future optimizations will be done to the ITC protocols to consider of the c-values generated by the high binding affinity of MetQ to L-methionine. To provide a range c-value from 10 – 1000. This includes doing a competitive binding study on wild-type MetQ with L-methionine

Acknowledgments & References

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1. Nguyen PT, Li QW, Kadaba NS, Lai JY, Yang JG, Rees DC.; The contribution of methionine to the stability of the Escherichia coli MetNIQ ABC transporter-substrate binding protein complex. Biol Chem. **2015,** 396, 1127-34.

2. Nguyen PT, Lai JY, Kaiser JT, Rees DC. Structures of the Neisseria meningitides methionine-binding protein MetQ in substrate-free form and bound to l- and d-methionine isomers. Protein Sci. **2019,** 10, 1750-1757.

3. Nguyen PT, Lai JY, Lee AT, Rees DC, Noncanonical role for the binding protein in substrate uptake by the MetNI methionine ATP Binding Cassette (ABC) transporter. *PNAS* **2018**, 115, E10596-E10604.

4. Turnbull WB, Daranas AH, On the Value of c: Can Low Affinity Systems Be Studied by Isothermal Titration Calorimetry? *J. Am. Chem. Soc.* **2003**, *125* (48), 14859-14866.