Quantification of reaction rates in the E. coli Min system

Steven S. Andrews, Adeleh Moghadam, and Jay T. Groves
Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA
Department of Chemistry, University of California, Berkeley, CA

Abstract
Cell division of E. coli bacteria occurs through a remarkable pole-to-pole oscillation of the proteins MinC, MinD, and MinE. Recently, this has become a popular system for computer simulation, leading to a wide variety of models that purport to explain the dynamics. To assist in the discrimination between proposed models, and to allow the development of more accurate models, we are experimentally measuring the rates of several of the biochemical reactions that comprise this system. This is done in vitro using purified lipids, proteins, and other chemicals. Experiments focus primarily on the rate of ATP hydrolysis by MinD. To extract information about different rates in the system from this single observable, chemical concentrations are varied widely between different analyses. This shifts the balances of fluxes in the in vitro reaction network, thus influencing the rate of ATP hydrolysis. The resulting multi-dimensional phosphate release data will be analyzed using computer models of the reaction network to yield individual reaction rate constants.

E. coli Min system introduction

• Used by the cell to position the division plane at the cell center.
• Dynamics depend on only two proteins, MinD and MinE.
• MinD and MinE oscillate from pole to pole with about a 40 second period.
• MinD forms a helical polymer on the inside of the cell membrane.
• MinD is an ATPase that is stimulated by MinE.

Why is the MinD polymer mostly at a pole and helical?

Shown in other work, the hypothesis of a co-author (SSA) is that it arises from mechanical interactions between the natural curvature of the polymer and the shape of the cell membrane. This theory leads to 5 polymer morphologies, of which MinD most closely resembles the “poly-targated” type.

How does MinD interact with the membrane?

Using cryo-electron microscopy, we find that MinD causes small unilamellar vesicles to deform, aggregate, and become bilamellar, even in the absence of ATP and Mg2+. Similar results are found using fluorescence microscopy of giant unilamellar vesicles.

Open questions with the Min system

• What is the Min system reaction network, including reaction rates?

This project is the topic of this poster.

The Min system reaction network

The reaction network structure is reasonably well known, but most rate constants and mechanisms are unknown.

Phosphate measurement with Malachite green

Every reaction affects release rate of inorganic phosphate (P_i) making this an observable that can be used to probe the reaction rates. We measure the phosphate production rate during in vitro reactions using purified components.

Concept
Malachite green reagent (BioAssay Systems) turns green upon addition of phosphate. This is quantified with A_655, internally referenced to A_550, on a plate reader. The color change is unstable, so it is monitored over 40 minutes.

Experimental method

In glass-bottom microplate well: mini MinD, ATP, MgCl_2, buffer, etc. React ~30 minutes.

Stop reaction with water, EDTA, and Malachite green (MG). MG color starts developing.

Add glass cover slip to microplate for flat top surface and put in plate reader.

Measure absorbance difference between 655 and 490 nm for 40 minutes.

Typical data

Data were analyzed by extrapolating Malachite green absorption data back to the end of the ATPase reaction, and rescaling with calibration standards. We are developing a method to fit complete kinetic traces with those from calibration standards to yield more accurate results.

Results

I. MinD reactions with ATP

Hydrolysis of ATP by MinD follows a mechanism similar to Michaelis-Menten, allowing similar analysis methods.

Experimental design:

Lineweaver-Burk plot of the reaction between ATP and MinD.

\[ v = \frac{V_{max} [ATP]}{K_m + [ATP]} \]

Intercept: [ATP] = 0

Slope : \[ V = \frac{V_{max}}{K_m} \]

K_m = 10^{-5} M

V \_max = 100 \_pm \_10^3 M \_s \_1

II. MinD activation by lipids and MinE

From literature results, lipids do not affect ATP hydrolysis (implying that k_2 is small), but the combination of lipids and MinE does. These speed up ATP hydrolysis by a factor of 10; accounting for the nucleotide transfer rates (k_1), the activated reaction rate constant is 16 times k_2.

k_2 < 0.5/[Mg2+] x s^{-1}

k_1 = 0.016 x s^{-1}

III. Mg2+ binding to MinD

MinD binds ATP with a Mg2+ ion. We find MinD is inactive without Mg2+ and rates with Mg2+ are fit by the Hill equation. The Hill coefficient of 2.0 implies that only MinD dimers hydrolyze ATP, which is supported by other experiments in which the MinD concentration was varied.

Conclusions

Quantification of phosphate release rates is a simple and powerful method for determining reaction rates in networks that involve ATP hydrolysis. Using an improved Malachite green method, we: (i) determined several reaction rates in the E. coli Min system, and (ii) found that only dimeric MinD is active.

In future work, we will rework the Min system reaction network to account for current results, quantify other reaction rates, and iterate until the in vitro network is fully quantified. This will require model-based parameter estimation methods.

Acknowledgements

The His-MinD strain was generously provided by Yu-ling Shih, in the Rothfield lab. The in vitro images were acquired in collaboration with Susan Rowland and Stephan Aung. Cryo-electron microscopy was performed in collaboration with Luis Comelli. We thank Adam Arkin, and the Arkin and Groves groups, for helpful discussions. Funding was provided by the US Department of Energy, a National Science Foundation postdoctoral fellowship in biological informatics awarded to SSA, and the University of California at Berkeley.