A gating model for the potassium efflux systems KefB and KefC

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1 Model Description

1.1 Motivations and Objectives

The activity of potassium efflux systems such as KefB and KefC in *Escherichia coli* is essential for survival of cells exposed to electrophiles. The formation of GSH adducts elicit the efflux of these systems while reduced GSH is known to maintain the inactive state. Mutational analysis has revealed the roles of key residues in the ligand-gated regulation of these systems [1].

The KefC efflux system has been extensively studied and documented. The crystal structure of its regulatory KTN domain has given insights on the gating mechanisms of such efflux systems [2]. KefB has 42% identity and 64% similarity to KefC. The two systems exhibit differences in their activation by various GSH adducts. For example, KefB is strongly activated by the glutathione (GSH) adducts S-lactoylglutathione (SLG) and N-ethylsuccinimido-S-glutathione (ESG), while KefC is only mildly activated by SLG and strongly activated by ESG. GSH-deficient mutants exhibit spontaneous leaks of K⁺, indicating the role of GSH as the ligand of the inactive conformation of these systems [3]. Experimental evidence of KefC oligomeric organisation together with the crystal structure of its cytoplasmic domain support the hypothesis that this system is a dimer [4].

We propose a kinetic model that describes the activation of a dimeric efflux system that could bind either GSH or SLG. The model is able to predict the distribution of possible channel states (Figure 1) when subjected to a fixed concentration of GSH and SLG. This model can be applied to other GSH adducts.
1.2 Assumptions

- The efflux system is a dimer, with two binding sites that can bind either GSH (G) or SLG (S).
- The system has 6 possible states: SS, SG=GS, GG, G0=0G, S0=0S and 00.
- State 00 (free efflux system) represents a leaking state.
- All possible open states exhibit the same ionic conductance.

2 Equations

System of differential equations:

\[
\begin{align*}
\frac{dSS}{dt} &= -2k_{-1}SS + k_{+1}(S0 + 0S) \\
\frac{dGG}{dt} &= k_{+2}(0G + G0) - 2k_{-2}GG \\
\frac{d00}{dt} &= k_{-1}(S0 + 0S) + k_{-2}(0G + G0) - 2(k_{+1} + k_{+2})00 \\
\frac{d(S0 + 0S)}{dt} &= 2k_{-1}SS - (k_{+1} + k_{+2} + k_{-2})(S0 + 0S) + k_{-2}(SG + GS) + 2k_{+1}00 \\
\frac{d(SG + GS)}{dt} &= k_{+2}(S0 + 0S) - (k_{-1} + k_{-2})(SG + GS) + k_{+1}(0G + G0) \\
\frac{d(0G + G0)}{dt} &= k_{-1}(SG + GS) - (k_{+1} + k_{-2} + k_{+2})(0G + G0) + 2k_{-2}GG + 2k_{+2}00
\end{align*}
\]

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_{1+}, k_{2+})</td>
<td>Binding of SLG and GSH respectively to the efflux system *</td>
</tr>
<tr>
<td>(k_{1-}, k_{2-})</td>
<td>Release of SLG and GSH respectively to the efflux system</td>
</tr>
</tbody>
</table>

Figure 1: Possible channel states and rate constants
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{d1} = \frac{k_{1-}[SLG]}{k_{1+}}$</td>
<td>Dissociation constant for SLG</td>
</tr>
<tr>
<td>$K_{d2} = \frac{k_{2-}[GSH]}{k_{2+}}$</td>
<td>Dissociation constant for GSH</td>
</tr>
</tbody>
</table>

*This is a first order rate constant. The dependence on SLG and GSH concentrations is implicit in the constants.

### 2.1 Analytical Solutions

<table>
<thead>
<tr>
<th>State</th>
<th>Equation (*N)</th>
</tr>
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<tbody>
<tr>
<td>SS</td>
<td>$\frac{[SLG]^2}{K_{d1}}$</td>
</tr>
<tr>
<td>GG</td>
<td>$\frac{[GSH]^2}{K_{d2}}$</td>
</tr>
<tr>
<td>00</td>
<td>1</td>
</tr>
<tr>
<td>S0 + 0S</td>
<td>$\frac{2[SLG]}{K_{d1}}$</td>
</tr>
<tr>
<td>SG + GS</td>
<td>$\frac{2[SLG][GSH]}{K_{d1}K_{d2}}$</td>
</tr>
<tr>
<td>G0 + 0G</td>
<td>$\frac{2[GSH]}{K_{d2}}$</td>
</tr>
</tbody>
</table>

Where $N$ is:

$$N = \left(1 + \frac{[SLG]}{K_{d1}} + \frac{[GSH]}{K_{d2}}\right)^2$$

### 3 Numerical Methods and Algorithm

Equilibrium solutions can be calculated analytically. Matlab 7.5 (version for Windows) was used to perform parameter estimation.

### 4 Computational Tools

A Windows XP Intel Core Duo computer was used to perform the parameter estimation simulations.
5 Parameter Estimation Techniques

Global Optimization Methods (Scatter Search Method) and Genetic Algorithms were used to estimate the parameters of the system. [5]

6 Applications and Limitations of the Model

- This model predicts the distribution of channel states depending on the fixed concentrations of SLG and GSH
- Cooperativity can be included by new assumptions on the binding and release constants
- Assumptions on the conductances of each state must be done for the analysis of the experimental data.

References


