Extending a dynamic model of trypanosome metabolism

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Overview

• **Introduction to *SilicoTryp***
  - Current model of glycolysis

• **Extension of model**
  - Trypanothione
  - Pentose phosphate pathway (PPP)
  - Wiki-page

• **Cytosolic PPP**

• **Glycosomal PPP**
  - Phosphate leak
  - Two hypotheses

• **Future extension**

• **Conclusions**
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To create a ‘Silicon Trypanosome’, a comprehensive, experiment-based, multi-scale mathematical model of trypanosome physiology.


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Trypanosoma brucei

- Protozoan parasite
- African sleeping sickness in human
- Nagana in cattle
- Fatal if untreated
- Drugs toxicity, difficulties in administration, emerging resistance
Glycolysis in Bloodstream Form *Trypanosoma brucei* Can Be Understood in Terms of the Kinetics of the Glycolytic Enzymes

(Received for publication, July 10, 1996, and in revised form, October 9, 1996)

Barbara M. Bakker, Paul A. M. Michels, Fred R. Opperdoes, and Hans V. Westerhoff

\[
\frac{[\text{Glc6P}]_g}{K_{m,\text{Glc6P}}} \cdot \frac{1 - \frac{[\text{Fru6P}]_g}{[\text{Glc6P}]_g \cdot K_{eq,\text{PGI}}}}{1 + \frac{[\text{Glc6P}]_g}{K_{m,\text{Glc6P}}} + \frac{[\text{Fru6P}]_g}{K_{m,\text{Fru6P}}}}
\]

\[
\frac{d[\text{Fru6P}]}{dt} = v_{\text{PGI}} - v_{\text{PFK}}
\]

- Part of glycolysis is localized in an unique peroxisome-like organelle: the glycosome
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- Oxidative stress protection
- Trypanothione = 2x glutathione + spermidine
- Eflornithine is suicide inhibitor of ornithine decarboxylase
- Pentose phosphate pathway provides NADPH

![Diagram of trypanothione pathway with drugs acting on it]
Pentose phosphate pathway in bloodstream form *T. brucei*
Phosphofructokinase

Chemical equation

\[
\text{Fructose-6-phosphate}_{\text{glycogen}} + \text{ATP}_{\text{glycogen}} \rightarrow \text{Fructose-1, 6-bisphosphate}_{\text{glycogen}} + \text{ADP}_{\text{glycogen}}
\]

Rate equation

\[
v_{PPK} = \frac{V_{\text{max}}}{K_{m,\text{Fru6P}} + \frac{K_i}{1 + \frac{Fru6P}{K_{m,\text{Fru6P}}} + \frac{Fr16BP}{K_{m,\text{ATP}}} + (1 + \frac{ATP}{K_{m,\text{ATP}}})}
\]

Parameters

\[V_{\text{max}} = 1708 \text{ nmol.min}^{-1}.\text{mg of cell protein}^{-1}\]

\[K_{m,\text{Fru6P}} = 0.82 \text{ mM}\]

The value was measured for the 2005 version of the model[1]; see supplementary information table S1. The assay is described in Misset and Opperdoes (1984)[2] (pH 7.6, T=25°C). The reported value is 1708±293 (average ± S.D. of n experiments)

From Cronn et al. (1985) [3]. The value was measured at pH=6.7 and reported in table 3:

<table>
<thead>
<tr>
<th>Conc. of enzyme (μg/ml)</th>
<th>V_{max} (nmol/min)</th>
<th>[Fructose-6-P]_4 (mM)</th>
<th>k</th>
</tr>
</thead>
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<tr>
<td>0.100</td>
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<td>1.2</td>
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<tr>
<td>0.010</td>
<td>267</td>
<td>1.00</td>
<td>1.3</td>
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<tr>
<td>0.005</td>
<td>267</td>
<td>1.26</td>
<td>1.3</td>
</tr>
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<td>0.003</td>
<td>567</td>
<td>1.90</td>
<td>1.4</td>
</tr>
</tbody>
</table>
Dynamic modelling under uncertainty:
The case of *Trypanosoma brucei* energy metabolism

Fiona Achcar
PS016
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Cytosolic pentose phosphate pathway
Extension with the cytosolic PPP: an (aspecific) phosphatase?

- Level of oxidative stress regulates flux through PPP
- Low oxidative stress leads to Glc6P accumulation
- Phosphatase activity would prevent this accumulation
- Initial experiments have confirmed this activity
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• **Further extension**

• **Conclusions**
Glycosomal pentose phosphate pathway
Extension with the glycosomal PPP: a 'phosphate leak'

- Glycosomal PPP introduces a 'phosphate leak'
- ATP/ADP balance in glycosome is perturbed
- Fluxes decline rapidly → non viable state
- Several hypotheses were tested
Hypothesis 1:
ATP:ADP antiporter

- Works at 5 mM glucose (blood)
- Glycosomal compartmentation of ATP is essential
- Accumulation of sugar phosphates at 25 mM glucose (culture medium)
- ATP:ADP antiporter mimicks absence of glycosome

Hypothesis 2: Ribokinase

- Ribokinase in reverse restores the ATP:ADP balance
- Ribose is produced from glucose
- Putative *T. brucei* ribokinase enzyme showed activity in both directions
- Knockdown (RNAi) and knockout experiments hint at essentiality

![Diagram of glycolysis and ribokinase pathways](image-url)
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Inhibition by ribose

- Model suggests sensitivity for ribose concentration
- 5 mM should be lethal
- Not reproducible in lab
- Not unexpected, as we know there are more pathways involved in ribose metabolism
**Trypanosoma brucei** biology:

- Phosphatase activity prevents accumulation of cytosolic glucose 6-phosphate
- Ribokinase restores phosphate balance in the glycosome

**Systems biology:**

- Even small extension of a well curated model brings up many questions
- Iterative cycle of experiments and modelling is necessary (and still continuing)
- Stress your model!
Acknowledgements

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