A Spoonful of Sugar: Metabolism and Glycolysis

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Introduction

Glycolysis is one of the fundamental processes of metabolism, but it is far from simple. It involves 11 elementary enzymatic reaction steps (see, for example, the book by Stryer[1]). The first three steps are

[1] L. Stryer. *Biochemistry*. W.H. Freeman, New York, 1988

1. phosphorylation of glucose to glucose 6-phosphate;
2. isomerization of glucose 6-phosphate to fructose 6-phosphate;
3. phosphorylation of fructose 6-phosphate to fructose 1,6-biphosphate.

The source of the phosphate groups for both the phosphorylation reactions is adenosine triphosphate (ATP), which ends up as adenosine diphosphate (ADP). However, another reaction involving adenosine monophosphate can also occur:

$$2\mathrm{ADP} \rightleftharpoons \mathrm{ATP} + \mathrm{AMP}.$$

As cells normally contain little AMP, this reaction tends to decrease the ATP/AMP ratio. What makes life interesting is that there is a feedback loop which affects these reactions. The third reaction is catalysed by the enzyme phosphofructokinase (PFK1), which is allosterically inhibited by ATP, but the inhibition is removed by AMP. So the greater the activity of PFK1, the more ADP is formed, so the more AMP is generated, so the more inhibition is removed from PFK1 – a positive feedback loop.

Simplified Model

In a simplified model of these processes, Sel'kov[2] proposed that the enzyme was directly activated by combining with γ molecules of ADP to form the complex $(\mathrm{PFK1}\diamondsuit\mathrm{ADP}^{\gamma})$, according to the scheme

[2] E E Sel'kov. Self-oscillations in glycolysis – 1. A simple kinetic model. *European Journal of Biochemistry*, 4(1):79–86, March 1968

$$\gamma\mathrm{ADP} + \mathrm{PFK1} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} (\mathrm{PFK1}\diamondsuit\mathrm{ADP}^{\gamma}).$$

Also, he assumed a steady supply of ATP, a complexing of ATP with the activated enzyme to release ADP, and an irreversible removal of ADP:

$$\begin{array}{c} \overset{\nu_1}{\rightarrow}\mathrm{ATP} \\ \mathrm{ATP} + (\mathrm{PFK1}\diamondsuit\mathrm{ADP}^{\gamma}) \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (\mathrm{ATP}\diamondsuit\mathrm{PFK1}\diamondsuit\mathrm{ADP}^{\gamma}) \overset{k_2}{\rightarrow} (\mathrm{PFK1}\diamondsuit\mathrm{ADP}^{\gamma}) + \mathrm{ADP} \\ \mathrm{ADP}\overset{\nu_2}{\rightarrow} \end{array}$$

By changing to dimensionless variables ($\sigma_1 = (k_1/(k_2 + k_{-1}))[\mathrm{ATP}]$, $\sigma_2 = (k_3/(k_{-3})^{1/\gamma}[\mathrm{ADP}]$, and scaling the concentrations of the complexes by the total amount of enzyme), and by assuming that the complexes respond so rapidly that their concentrations may be replaced by their steady-state values[3], the equations can be rewritten as

[3] Work through these changes, using Mathematica to assist you.

$$f(\sigma_1, \sigma_2) = \frac{\sigma_1 \sigma_2^\gamma}{\sigma_1 \sigma_2^\gamma + \sigma_2^\gamma + 1} \tag{1}$$

$$\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} = \nu - f(\sigma_1, \sigma_2) \tag{2}$$

$$\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} = \alpha f(\sigma_1, \sigma_2) - \eta\sigma_2. \tag{3}$$

Begin by studying numerically the behaviour of these equations, including the effects of the parameters and the initial conditions. One interesting set of results can be obtained with the parameters $\nu = 0.0285$, $\eta = 0.1$, $\alpha = 1.0$ and $\gamma = 2.0$ and with initial conditions $\sigma_1(0) = 0.3$ and $\sigma_2(0) = 0.3$. A useful range of τ is from 0 to 1000. In your investigations use plots of σ_1 and σ_2 as functions of the time parameter τ as well as phase portraits in which σ_2 is plotted against σ_1 with τ as a parameter.

You might try to solve the full set of differential equations, without the steady-state assumptions, to see how fast the equilibration has to be for the steady-state assumption to be valid.

More Detailed Model

Unfortunately, this is not yet the full story. Hess and Boiteux[4] found that there were stable steady-state systems for both high and low injection rates of ATP. To describe this, Goldbeter and Lefever[5] proposed a more complex model of Monod-Wyman-Changeux type, as follows[6].

[4] B Hess and A Boiteux. Substrate control of glycolytic oscillations. In B Chance, E K Pye, A K Ghosh, and B Hess, editors, *Biological and Biochemical Oscillators*. Academic Press, 1973

[5] A Goldbeter and R Lefever. Dissipative structures for an allosteric model: application to glycolytic oscillations. *Biophysical Journal*, 12(10):1302–1315, October 1972

[6] Brace yourself!

The enzyme PFK1 is assumed to be a dimer which exists in two states, active (R) and inactive (T). The ATP substrate S_1 can bind to both forms, but the ADP product, S_2, is an activator of the enzyme and only binds to the active form. So the species we need to consider are inactive forms of the enzyme bound to j molecules of ATP, which we label T_j, and the active form of the enzyme bound to i molecules of ATP and j molecules of ADP, labelled as R_{ij}. The reactions are shown in Figure 1: the factor of two in some of the reaction rates represents the availability of two binding sites.

Again convert to a dimensionless form ($\sigma_1 = (k_2/k_{-2})s_1$, $\sigma_2 = (k_2/k_{-2})s_2$) and use steady-state assumptions, and with the aid of Mathematica reduce the equations to a form similar to Equations 2 and 3, but with a different function f.

Investigate the behaviour of the new system, studying in particular whether it exhibits different behaviour from the simpler model.

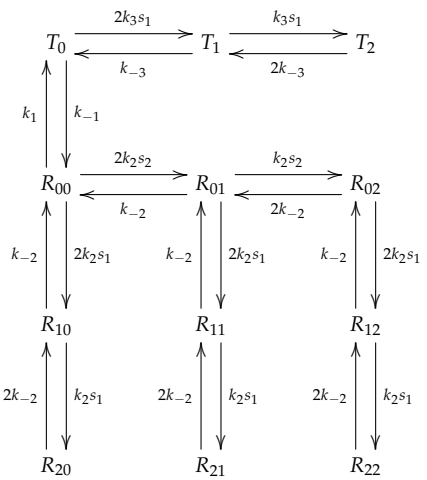

Figure 1: Receptor states in the Goldbeter-Lefever model of glycolysis.

Possible Extensions

There are two ways in which this investigation can be taken further if time permits. The first is to apply analytical methods to find the *nullclines* of the systems, to locate the steady-state solution, and to investigate the stability of the steady-state solution. Alternatively, there are various simplifications of the Goldbeter-Lefever model (for example, that of Smolen[7]), and the project can be extended to investigate one or more of those.

[7] Paul Smolen. A model for glycolytic oscillations based on skeletal muscle phosphofructokinase kinetics. *Journal of Theoretical Biology*, 174(2):137 – 148, 21 May 1995

References

[1] A Goldbeter and R Lefever. Dissipative structures for an allosteric model: application to glycolytic oscillations. *Biophysical Journal*, 12(10):1302–1315, October 1972.

[2] B Hess and A Boiteux. Substrate control of glycolytic oscillations. In B Chance, E K Pye, A K Ghosh, and B Hess, editors, *Biological and Biochemical Oscillators*. Academic Press, 1973.

[3] E E Sel'kov. Self-oscillations in glycolysis – 1. A simple kinetic model. *European Journal of Biochemistry*, 4(1):79–86, March 1968.

[4] Paul Smolen. A model for glycolytic oscillations based on skeletal muscle phosphofructokinase kinetics. *Journal of Theoretical Biology*, 174(2):137 – 148, 21 May 1995.

[5] L. Stryer. *Biochemistry*. W.H. Freeman, New York, 1988.

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