Modelling Biochemical Reaction Networks

Lecture 10: Glycerol metabolism, Part II

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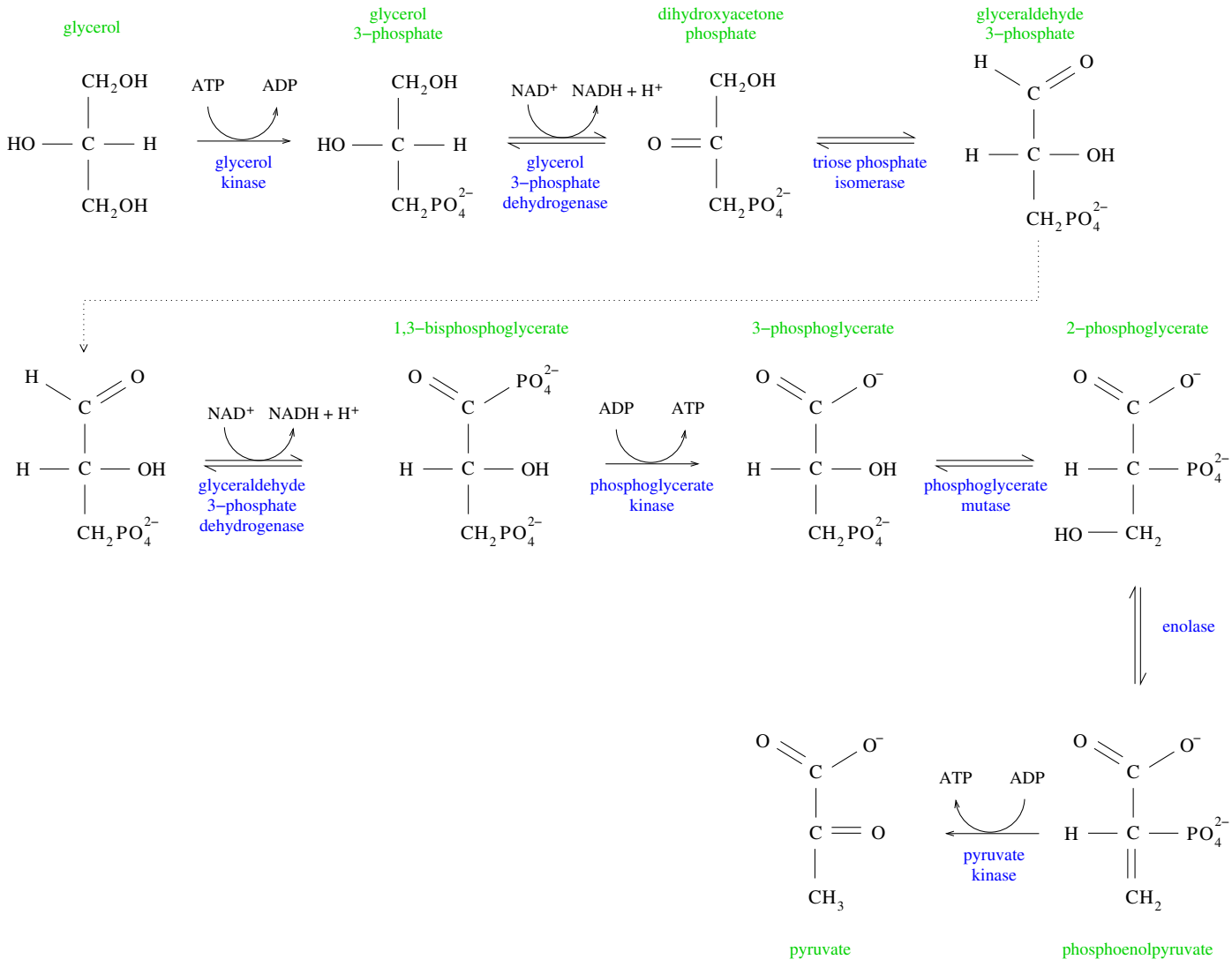
SBML: Systems Biology Markup Language

- A standardized computer-readable format for representing biochemical models
- Allows a specification of rate laws, parameters and their units, compartments, chemical species, reactions, etc.
- Example SBML model:
  `http://sbml.org/More_Detailed_Summary_of_SBML`
- Many computer programs are designed to create and work with SBML models without you having to know how to do it by hand.
  $\Longrightarrow$ data interchange format
- Many models available in a searchable database:
  `http://www.ebi.ac.uk/biomodels-main`
- This database can generate `xppaut` input files for an SBML model.

Borrowing from the literature

- For standard pathways like glycolysis, we can often find a literature (possibly SBML) model where someone else has done the work collecting parameters, working out the ODEs, etc.
- Strategy: Look for a model that contains as much of the relevant pathways as possible, and add whatever is necessary from there.
- May need to also delete irrelevant reactions

Model


glycerol
glycerol 3–phosphate
dihydroxyacetone phosphate
glyceraldehyde 3–phosphate
ATP
ADP
glycerol kinase
NAD+
NADH + H+
glycerol 3–phosphate dehydrogenase
triose phosphate isomerase
1,3–bisphosphoglycerate
3–phosphoglycerate
2–phosphoglycerate
glyceraldehyde 3–phosphate dehydrogenase
phosphoglycerate kinase
phosphoglycerate mutase
enolase
pyruvate kinase
pyruvate
phosphoenolpyruvate

Model of Hynne and Sørensen

Biophys. Chem. **94**, 121 (2001).

- This model of glycolysis in *Saccharomyces cerevisiae* has most of the reactions we need, and several we don't.
- Get `xppaut` code, and prune out unnecessary stuff.
- For species considered constant in our model, replace `init` (initial condition) statements by `param` and delete differential equation.
  Examples: ATP, extracellular glycerol
- Delete all references to sink (pyruvate).
- Model contains rate for "glycerol synthesis", i.e. the reaction

  $$\text{dihydroxyacetone phosphate} + \text{NADH} \rightarrow \text{glycerol} + \text{NAD}^+$$

  This is the reverse of what we want.
- Add glycerol kinase and glycerol 3-phosphate dehydrogenase reactions.

Glycerol 3-phosphate dehydrogenase

- Catalyzes the reaction

$$\mathrm{Gol3P} + \mathrm{NAD}^+ \rightleftharpoons \mathrm{DHAP} + \mathrm{NADH}$$

(Gol3P=glycerol 3-phosphate; DHAP=dihydroxyacetone phosphate)

- The rate of the reverse reaction, which dominates under most conditions *in vivo*, has been studied extensively and obeys the equation

$$v_{\mathrm{g3pd,rev}} = v^{(\mathrm{rev})}_{\mathrm{max,g3pd}}[\mathrm{NADH}][\mathrm{DHAP}] \Big/ \Big\{ K_b \left(1 + [\mathrm{P}_i]/K_{P,\mathrm{g3pd}}\right) \left[[\mathrm{NADH}] + K_{ia}\left(1 + [\mathrm{NAD}^+]/K_{iq}\right)\right] + [\mathrm{DHAP}]\left[[\mathrm{NADH}] + K_a\left(1 + [\mathrm{NAD}^+]/K_{iq}\right)\right]\Big\}$$

Cai et al., J. Biotech. **49**, 19 (1996).

Glycerol 3-phosphate dehydrogenase

- Little is known about the kinetics of the forward reaction.
- We do however know the equilibrium constant for the reaction:

$$K_{\text{eq}} = \frac{[\text{DHAP}][\text{NADH}]}{[\text{Gol3P}][\text{NAD}^+]} = 2.9 \times 10^{-5}$$

Cai et al., J. Biotech. **49**, 19 (1996).

Glycerol 3-phosphate dehydrogenase

- Because we're treating NADH and NAD^+ as constant, the rate law for the reverse reaction reduces to

$$v_{\text{g3pd,rev}} = \frac{v'_{\text{max,g3pd}}[\text{DHAP}]/K_{\text{g3pd,DHAP}}}{1 + [\text{DHAP}]/K_{\text{g3pd,DHAP}}}$$

where

$$v'_{\text{max,g3pd}} = \frac{v^{(\text{rev})}_{\text{max,g3pd}}[\text{NADH}]}{[\text{NADH}]+K_a\left(1+[\text{NAD}^+]/K_{iq}\right)}$$

$$K_{\text{g3pd,DHAP}} = \frac{K_b\left(1+[\text{P}_i]/K_{P,\text{g3pd}}\right)\left[[\text{NADH}]+K_{ia}\left(1+[\text{NAD}^+]/K_{iq}\right)\right]}{[\text{NADH}]+K_a\left(1+[\text{NAD}^+]/K_{iq}\right)}$$

Glycerol 3-phosphate dehydrogenase

- Cai et al. (1996) recovered 1.5 mg of glycerol-3-phosphate dehydrogenase from 30 g of cells, with a yield of 43%. Assuming that the density of a cell is about 1 g/mL,

$$c_{\text{g3pd}} = \frac{1.5\,\text{mg}}{30 \times 10^{-3}\,\text{L}} \times \frac{1}{0.43} = 116\,\text{mg/L}$$

- Cai et al. (1996) also give a specific activity of $55.0\,\mu\text{mol}\,\text{min}^{-1}\text{mg}^{-1}$, from which we calculate

$$\begin{aligned} v^{(\text{rev})}_{\text{max,g3pd}} &= (116\,\text{mg/L})(55.0\,\mu\text{mol}\,\text{min}^{-1}\text{mg}^{-1}) \\ &= 6395\,\mu\text{M}\,\text{min}^{-1} \equiv 6.4\,\text{mM}\,\text{min}^{-1} \end{aligned}$$

- Hynne and Sørensen's model has [NADH] = 0.33 mM, $[\text{NAD}^+] = 0.65\,\text{mM}$.
  K_a and K_{iq} given by Cai et al. (1996).
- Calculate $v'_{\text{max,g3pd}} = 1.9\,\text{mM}\,\text{min}^{-1}$

Glycerol 3-phosphate dehydrogenase

- Cai et al. (1996) also give K_b, $K_{P,g3pd}$, K_{ia} and K_{iq}.
- $[\mathrm{P}_i] = 22\,\mathrm{mM}$ (Albe et al., J. Theor. Biol. **143**, 163, 1990)
- Calculate: $K_{\mathrm{g3pd,DHAP}} = 24\,\mathrm{mM}$

Interlude: Rate law for the reversible Michaelis-Menten mechanism

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{C} \underset{k_2}{\overset{k_{-2}}{\rightleftharpoons}} \mathrm{E} + \mathrm{P}$$

- Apply enzyme conservation and the steady-state approximation:

$$\frac{dC}{dt} = k_1 S(E_0 - C) - (k_{-1} + k_{-2})C + k_2 P(E_0 - C) \approx 0$$

$$\therefore C = \frac{E_0(k_1 S + k_2 P)}{k_1 S + k_2 P + k_{-1} + k_{-2}}$$

Interlude

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{C} \underset{k_2}{\overset{k_{-2}}{\rightleftharpoons}} \mathrm{E} + \mathrm{P}$$

$$\begin{aligned} v = \frac{dP}{dt} &= k_{-2}C - k_2 P(E_0 - C) \\ &= \frac{k_1 k_{-2} E_0 S - k_{-1} k_2 E_0 P}{k_1 S + k_2 P + k_{-1} + k_{-2}} \\ &= \frac{v^+_{\max} S/K_S - v^-_{\max} P/K_P}{1 + \frac{S}{K_S} + \frac{P}{K_P}} \end{aligned}$$

where

$$v^+_{\max} = k_{-2}E_0 \qquad K_S = (k_{-1} + k_{-2})/k_1$$
$$v^-_{\max} = k_{-1}E_0 \qquad K_P = (k_{-1} + k_{-2})/k_2$$

Back to glycerol 3-phosphate dehydrogenase

- Compare

$$v = \frac{v^+_{\mathrm{max}} S/K_S - v^-_{\mathrm{max}} P/K_P}{1 + \frac{S}{K_S} + \frac{P}{K_P}}$$

  and

$$v_{\mathrm{g3pd,rev}} = \frac{v'_{\mathrm{max,g3pd}}[\mathrm{DHAP}]/K_{\mathrm{g3pd,DHAP}}}{1 + [\mathrm{DHAP}]/K_{\mathrm{g3pd,DHAP}}}$$

- In our case, $P = [\mathrm{DHAP}]$, and $S = [\mathrm{Gol3P}]$; $v^-_{\mathrm{max}} = v'_{\mathrm{max,g3pd}}$, $K_P = K_{\mathrm{g3pd,DHAP}}$.
- Cai et al. (1996) give $K_S = K_{\mathrm{Gol3P}} > 50\,\mathrm{mM}$.
  Another isoform of the enzyme has $K_{\mathrm{g3pd,Gol3P}} = 34\,\mathrm{mM}$ (Påhlman et al., J. Biol. Chem. **277**, 27991, 2002).
  Use $K_{\mathrm{g3pd,Gol3P}} = 34\,\mathrm{mM}$.

Back to glycerol 3-phosphate dehydrogenase

- At equilibrium, $v = 0$, so

$$v^+_{\text{max}} S/K_S = v^-_{\text{max}} P/K_P$$

$$\therefore \frac{P}{S} = \frac{v^+_{\text{max}} K_P}{v^-_{\text{max}} K_S} \qquad \text{(Haldane relation)}$$

- In our case,

$$\frac{[\text{DHAP}]}{[\text{Gol3P}]} = 2.9 \times 10^{-5} \frac{[\text{NAD}^+]}{[\text{NADH}]} = 5.7 \times 10^{-5}$$

- Solving for v^+_{max}, we get

$$v^+_{\text{max}} = (5.7 \times 10^{-5}) \frac{(1.9\,\text{mM/min})(34\,\text{mM})}{50\,\text{mM}} = 1.5 \times 10^{-4}\,\text{mM/min}$$

Glycerol 3-phosphate dehydrogenase

Summary:

$$v_{\mathrm{g3pd}} = \frac{v^{+}_{\mathrm{max,g3pd}}[\mathrm{Gol3P}]/K_{\mathrm{g3pd,Gol3P}} - v^{-}_{\mathrm{max,g3pd}}[\mathrm{DHAP}]/K_{\mathrm{g3pd,DHAP}}}{1 + [\mathrm{Gol3P}]/K_{\mathrm{g3pd,Gol3P}} + [\mathrm{DHAP}]/K_{\mathrm{g3pd,DHAP}}}$$

with

$$v^{+}_{\mathrm{max,g3pd}} = 1.5 \times 10^{-4}\,\mathrm{mM/min} \qquad K_{\mathrm{g3pd,Gol3P}} = 34\,\mathrm{mM}$$

$$v^{-}_{\mathrm{max,g3pd}} = 1.9\,\mathrm{mM/min} \qquad K_{\mathrm{g3pd,DHAP}} = 24\,\mathrm{mM}$$