Modelling Biochemical Reaction Networks

Lecture 9: Glycerol metabolism, Part I

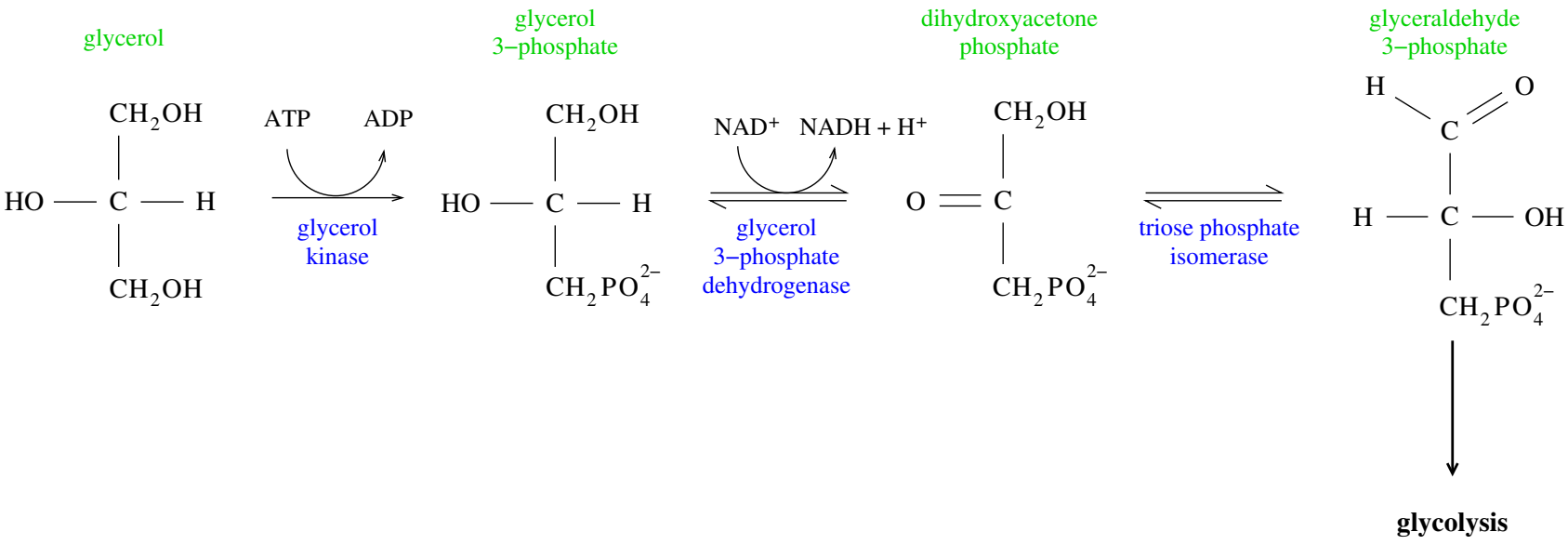
Marc R. Roussel

Department of Chemistry and Biochemistry

University of Lethbridge

Glycerol metabolism

- Glycerol is one of the building blocks of lipids.
- Used as an energy source by conversion to a form that can be injected into the glycolytic pathway:



Flux through a pathway

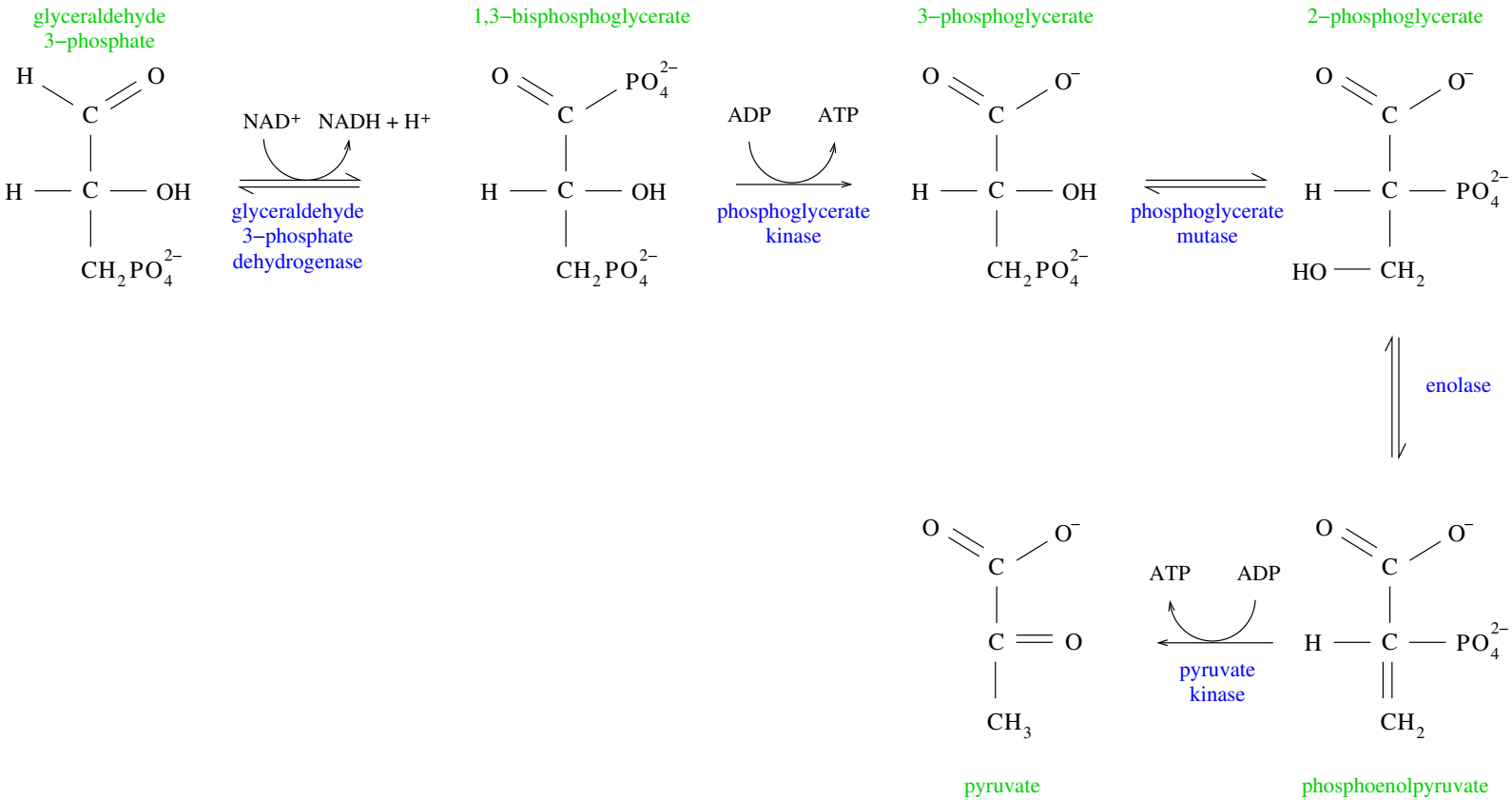
- Rate at which material "moves through" a pathway
- To define a flux, need a "source" and a "sink"
- Options for a source:
  - Constant glycerol
  - Constant rate of addition of glycerol
- Options for a sink:
  - Neglect reversibility of triose phosphate isomerase and make D-glyceraldehyde 3-phosphate the sink
  - Include one or more reactions from glycolysis, the last of which is irreversible (in reality or by assumption)

Questions

- Glycerol is a byproduct of various industrial processes (production of soap, biodiesel, vegetable oil).
- We might want to use it as a feedstock for production of (e.g.) yeast, for baking, brewing/fermenting, or sometimes used as nutritional supplements for cattle.
- What factor(s) limit the flux through this pathway?
- Can we engineer a strain of *Saccharomyces cerevisiae* that is capable of a higher flux through this pathway?

Glycolysis "payoff phase"

- We have to be careful not to "choke" glycolysis, so we should model the relevant part of this pathway, the so-called "payoff phase":



Cosubstrates

- Several reactions have cosubstrates (ATP, ADP, NAD^+, etc.).
- Treat as constant using typical *in vivo* values
- Resource: K. R. Albe et al., J. Theor. Biol. **143**, 163 (1990).
- Must know rate law, which depends on order of binding and other details
- Issue can sometimes be ducked, depending on how parameters were measured

Locating enzyme parameters

- We need (a) rate law, (b) K_M for each substrate, and (c) v_{max} or (d) k_{cat} and $[\text{E}]_{\text{total}}$ ($v_{\text{max}} = k_{\text{cat}}[\text{E}]_{\text{total}}$).
- Preferably need parameters for each enzyme from our target organism
- Useful resource: BRENDA, a database of enzyme kinetic parameters (`http://www.brenda-enzymes.org`)

  Example: glycerol kinase

Estimating the kinetic parameters of glycerol kinase in S. cerevisiae

- K_M(glycerol) = 2 mM [C. C. Aragon et al., J. Mol. Catal. B **52–53**, 113 (2008)]
- BRENDA gives values of the turnover number (k_{cat}) and of the specific activity (v_{max}/c_E, where c_E is the concentration of enzyme in g/L)
  - Either way, need enzyme concentration to get v_{max}
  - No values given for *S. cerevisiae*

Estimating the kinetic parameters of glycerol kinase in S. cerevisiae

- It would be unusual to measure a K_M without also obtaining a $v_{\max}$, so go look at Aragon et al. (2008).
  - $v_{\max} = 1.15\,\mathrm{U/mL}$
  - Methods, section 2.5: "One unit (U) of enzyme was defined as the amount of the enzyme catalyzing the formation of 1 μmol of glycerol-3-phosphate/min at 60°C."
  - $v_{\max} = 1.15\,\mu\mathrm{mol\,(mL)^{-1}min^{-1}} \equiv 19.2\,\mu\mathrm{mol\,L^{-1}s^{-1}}$

Problem: Data given at 60°C, not the 20–30°C of industrial processes

Rule of thumb: Rate constants approximately double for every 10°C increase in temperature

  - $v_{\max}$ at 20°C should be about 2^4 times smaller than at 60°C, or about $1\,\mu\mathrm{mol\,L^{-1}s^{-1}}$.

Estimating the kinetic parameters of glycerol kinase in S. cerevisiae

ATP as cosubstrate

- Issue not addressed by Aragon et al. (2008)
- Assays carried out in presence of a roughly physiological concentration of ATP (2.6 mM, somewhat higher than the 1–2 mM usually found in yeast; Albe et al., 1990)
- Get effective rate law for that concentration of ATP
- Given uncertainties in other parameters, this should be OK.

Estimating the kinetic parameters of glycerol kinase in S. cerevisiae

Summary

$$v_{gk} = \frac{v_{\max}[\text{glycerol}]}{K_{gk} + [\text{glycerol}]}$$

with

$$v_{\max} = 1\,\mu\text{mol}\,\text{L}^{-1}\text{s}^{-1}$$
$$K_{gk} = 2\,\text{mM}$$

Next time

- We could continue in this vein, and in some cases we have no other choice.
- Next time: another key resource that allows us to build on other people's work