Introduction on metabolism & refresher in enzymology

Daniel Kahn
Laboratoire d'Ecologie Microbienne
Lyon 1 University
& INRA MIA Department

Daniel.Kahn@univ-lyon1.fr

General objectives of the course

- Understand the general behaviour of metabolic systems
- Ability to model their dynamics
- Express how kinetic enzyme properties affect metabolite concentrations and fluxes
- Express how networks respond to changes in environment
- Understand the bases of Flux Balance Analysis
- Examine how experimental data may be used to identify a metabolic model
- Interpret these behaviours in terms of biological regulation
- Generalize to signal transduction networks

Course prerequisites

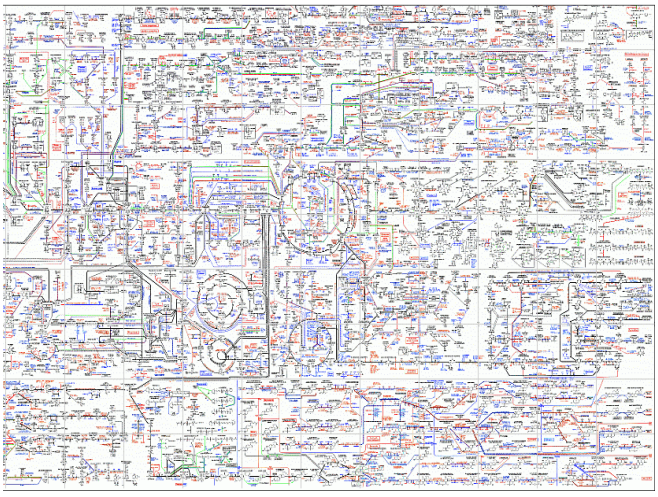
- Knowledge of enzyme kinetics
- Linear algebra
  - Matrix rank analysis, diagonalization, etc…
  - Familiarity with a mathematical package such as Scilab, Maple, R or Matlab
- Dynamical systems
  - Jacobian
  - Stability analysis

Outline

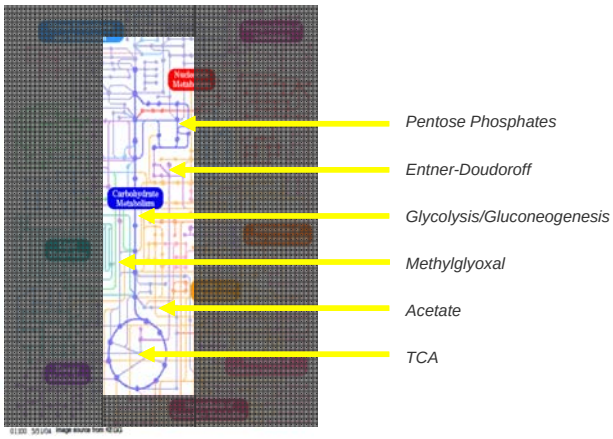
1. Introduction on metabolism
2. Methods to investigate metabolism
3. Refresher of enzyme kinetics

1. What is metabolism?

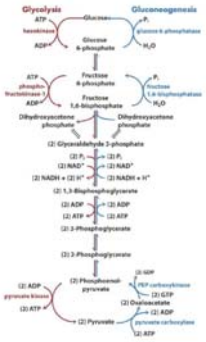
- Life's chemical factory
  - Typically several hundred reactions involving small molecules
- Balances
  - Nutrients and outputs
  - Energy
  - Redox…
- Fast turnover
- Almost always catalyzed by enzymes



Central C metabolism subnetwork

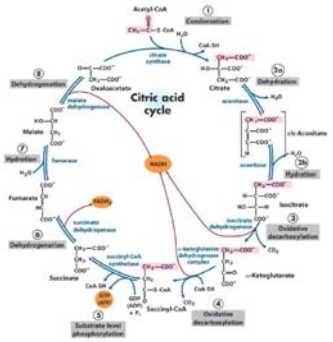


Glycolysis / gluconeogenesis



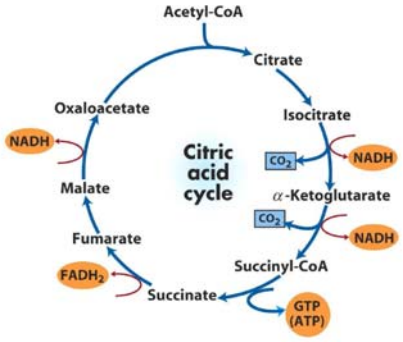
D. Kahn, Introduction on metabolism & refresher in enzymology

TCA cycle



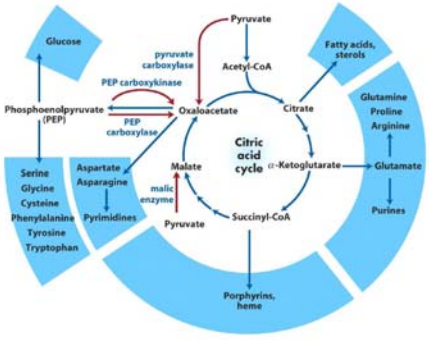
D. Kahn, Introduction on metabolism & refresher in enzymology

TCA cycle



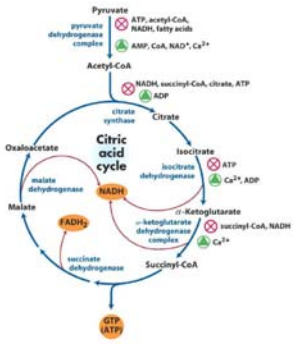
D. Kahn, Introduction on metabolism & refresher in enzymology

Anaplerosis



D. Kahn, Introduction on metabolism & refresher in enzymology

Regulation

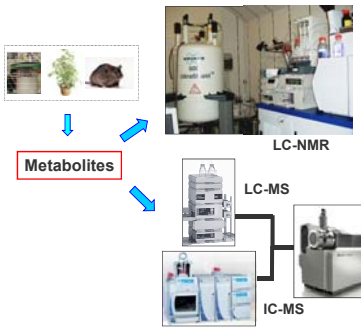


D. Kahn, Introduction on metabolism & refresher in enzymology

2. Methods to investigate metabolism

- Metabolomics: metabolite identification and quantitation
- Fluxomics
- Analytical tools based on
  - Nuclear Magnetic Resonance (NMR)
  - Mass spectrometry (MS)
  - Liquid chromatography (LC)

Metabolomics



NMR
- Complex mixtures
- Identification / Structure

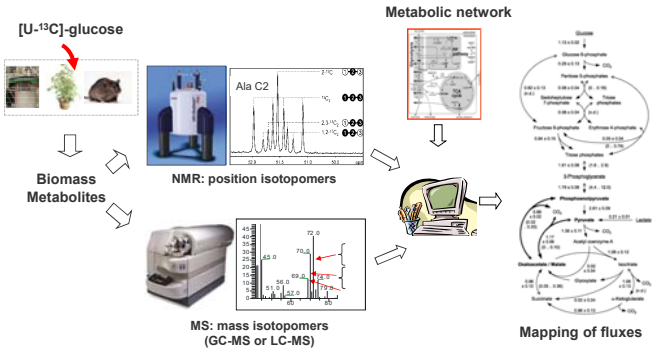
LC-NMR
- Targetted
- Suitable for high throughput

MS/MS

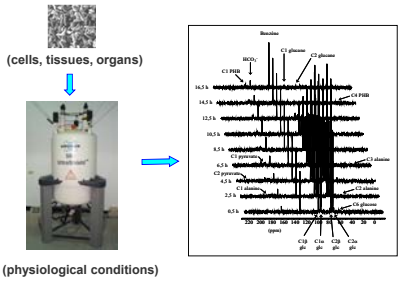
LC-MS/MS
Lipids, sugars, organic acids, aminoacids, coA esters…

IC-MS/MS
Sugar phosphates, nucleotides, organic acids…

Flux measurements



In vivo NMR



^{13}C
Carbon distribution
In vivo dynamics

^{31}P
Energy metabolism
pH, compartmentation
etc.

^{15}N
Nitrogen metabolism
N/C metabolic coupling

3. Enzyme kinetics: Michaelis-Menten

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

Mass action kinetics:

$v_1 = k_1 E.S - k_{-1} ES$

$v_2 = k_2 ES$

Quasi steady-state:

$v_1 = v_2 = v$

$E + ES = E_0$

Michaelis-Menten

Michaelis-Menten

E + S ⇌ ES → E + P

$$v = E_0 \frac{k_E S}{1 + \frac{S}{K_m}}$$ reaction rate $(M.s^{-1})$

$K_m = \frac{k_{-1} + k_{cat}}{k_1}$ Michaelis constant (M)

$k_E = \frac{k_{cat}}{K_m}$ catalytic efficiency $(M^{-1}.s^{-1})$

k_{cat} is the maximal turnover rate (s^{-1})

Reversible Michaelis-Menten

E + S ⇌ ES ⇌ E + P

$$v = E_0 \frac{k_+ S - k\_P}{1 + \frac{S}{K_S} + \frac{P}{K_P}}$$

This is the default expression for kinetic modelling, even when $k\_ = 0$, because it also accounts for competitive product inhibition.

Competitive inhibition

E + S ⇌ ES ⇌ E + P

E.I E.I

$$v = E_0 \frac{k_+ S - k\_P}{1 + \frac{S}{K_S} + \frac{P}{K_P} + \frac{I}{K_{Ic}}}$$

Other inhibitions

- Uncompetitive (more effective at high substrate concentration)

$$v = E_0 \frac{k_+ S - k\_P}{1 + \left(\frac{S}{K_S} + \frac{P}{K_P}\right)\left(1 + \frac{I}{K_{Iu}}\right)}$$

- Mixed

$$v = E_0 \frac{k_+ S - k\_P}{1 + \left(\frac{S}{K_S} + \frac{P}{K_P}\right)\left(1 + \frac{I}{K_{Iu}}\right) + \frac{I}{K_{Ic}}}$$

Multiple substrates and products

If substrates and products bind independently and in random order:

$$v = E_0 \frac{k_{cat}^{+} \prod_i \frac{S_i}{K_{S_i}} - k_{cat}^{-} \prod_j \frac{P_j}{K_{P_j}}}{\prod_i \left(1 + \frac{S_i}{K_{S_i}}\right) + \prod_j \left(1 + \frac{P_j}{K_{P_j}}\right) - 1}$$

'Convenience kinetics'

Liebermeister & Klipp, 2006, *Theoret. Biol. Med. Mod.* **3**:41

Haldane relationships

Equilibrium constraint:

$$K_{eq} = \frac{k_{cat}^{+}}{k_{cat}^{-}} \frac{\prod_j K_{P_j}}{\prod_i K_{S_i}}$$

Enzyme kinetics & thermodynamics

If we call Γ the mass action ratio

$$\Gamma = \frac{\prod_j P_j}{\prod_i S_i} = K_{eq} \exp\left(\Delta G' / RT\right)$$

Enzyme kinetics can be separated into three terms :

$$v = k_{cat}^{+} E_0 \,.\, f(S_i, P_j) \,.\, \left(1 - \Gamma / K_{eq}\right)$$

Enzyme kinetics & thermodynamics

$$v = k_{cat}^{+} E_0 \,.\, f(S_i, P_j) \,.\, \left(1 - \Gamma / K_{eq}\right)$$

- The first term $k_{cat}^{+} E_0$ corresponds to enzyme capacity
- The second term $0 < f(S_i, P_j) < 1$ is an enzyme saturation term.
  As an exercise, write this term for reversible Michaelis-Menten kinetics.
- The third term is a purely thermodynamic term, independent of enzyme properties:

$$1 - \Gamma / K_{eq} = 1 - \exp\left(\Delta G' / RT\right)$$

Cooperativity

Hill equation:

$$v = E_0 \frac{k_{cat} (S / K_{0.5})^h}{1 + (S / K_{0.5})^h}$$

h is the Hill coefficient. Typically : $0.5 < h < 4$

This equation is purely empirical
(actually it is wrong for $S << K_{0.5}$)

$K_{0.5}$ is a phenomenological constant (not a K_m)

2-site cooperative binding

Adair equation

$$v = 2 E_0 k_{cat} \frac{S / K_1 + S^2 / K_1 K_2}{1 + 2S / K_1 + S^2 / K_1 K_2}$$

realistically captures site dependencies

Saturation

E + S $\rightleftharpoons$ ES

$$E + ES = E_0$$

$$\frac{E.S}{ES} = \frac{k_{-1}}{k_1} = K_d$$

$$Y = \frac{ES}{E_0} = \frac{S / K_d}{1 + S / K_d}$$ saturation coefficient

Further reading

- *Understanding the Control of Metabolism*, by David Fell Portland Press, London, 1997
- *Fundamentals of Enzyme Kinetics*, by Athel Cornish-Bowden Portland Press, London, 2004