TOWARDS A THEORY OF METABOLISM

METABOLISM MODELLING

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Cours metabolisme - 1
THERE EXISTS A THEORY OF METABOLISM BASED ON:

I – Enzyme kinetics.

II – Structure of metabolic networks – Stoichiometry Matrix.


IV – Metabolic control theory.

V – Metabolic regulations.

VI – Genetic regulations.

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I

INTRODUCTION

CELLULAR METABOLISM
THE CELL AS AN OPEN SYSTEM

Sugars, Proteins, FAT

Energy (ATP) + Small molecules

Degradations (catabolism)

Biosyntheses (anabolism)

Macromolecules

2 cells

O₂, light,…

CO₂ + H₂0

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BASIC MOLECULES WITH MOLECULAR WEIGHT

Glucose (180)

Lysine (146,19)

ATP (507)

NADH (741)

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E.coli: ≈ 750 reactions and ≈ 500 metabolites

*In silico* predictions of *Escherichia coli* metabolic capabilities are consistent with experimental data. Jeremy S. Edwards, Rafael U. Ibarra & Bernhard O.

*Nature Biotechnology* 19, 125 - 130 (2001)
Metabolism Map
(from: Kyoto Encyclopedia of Genes and Genomes www.genome.ad.jp/kegg)

In this map, each dot represents an intermediate; each line represents an enzyme that acts on an intermediate.
Interconnections of metabolic reactions
Each metabolic step is catalyzed by an enzyme.
DIMENSION OF ENZYMES AND THEIR METABOLITES

Enzyme MW = 50 000

Metabolite MW = 100

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<table>
<thead>
<tr>
<th>Dimension</th>
<th>PM</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>nm (=10^-9 m)</td>
<td>1 Å = 10^-8 cm = 10^-10 m = 0,1 nm</td>
<td></td>
</tr>
<tr>
<td>Water (H2O)</td>
<td>0.26</td>
<td>18</td>
</tr>
<tr>
<td>Alanine (Amino acid)</td>
<td>0.5</td>
<td>89</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.7</td>
<td>180</td>
</tr>
<tr>
<td>ATP</td>
<td>1.6</td>
<td>507</td>
</tr>
<tr>
<td>NADH</td>
<td>741</td>
<td>Dipotassium salt</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>3.5</td>
<td>750</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>3.6</td>
<td>16 900</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>6.8</td>
<td>65 000</td>
</tr>
<tr>
<td>Cytochrome b</td>
<td>42 592</td>
<td>Part of complex of respiratory chain in mitochondria</td>
</tr>
<tr>
<td>Complex III</td>
<td>7x7x11</td>
<td>240 000</td>
</tr>
<tr>
<td>Ribosome (E.coli)</td>
<td>18</td>
<td>2 800 000</td>
</tr>
<tr>
<td>Membrane</td>
<td>4</td>
<td>Thickness</td>
</tr>
<tr>
<td>Lysosome</td>
<td>250-500</td>
<td></td>
</tr>
<tr>
<td>Peroxisome</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>Mitochondrion</td>
<td>1 500</td>
<td>1.5 pg</td>
</tr>
<tr>
<td>E. coli</td>
<td>2 000</td>
<td>2 pg</td>
</tr>
<tr>
<td>Nucleus</td>
<td>4 – 6 000</td>
<td></td>
</tr>
<tr>
<td>Chloroplast</td>
<td>8 000</td>
<td>60 pg</td>
</tr>
<tr>
<td>Hepatic cell</td>
<td>20 000</td>
<td>8 000 pg</td>
</tr>
</tbody>
</table>

507 = PM of acid form. PM of Na salt = 573

Small protein

Medium size protein

Large protein

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SOME PRESUPPOSITIONS

- The cellular medium is homogeneous.

- The cell metabolism works at steady state: \( \frac{d[X_i]}{dt} = 0 \).

- One deals with populations of molecules great enough to speak of concentrations (necessary to ODE approach).
II

ENZYME KINETICS

AN OVERVIEW

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Mass action modelling

\[ E + S \xrightleftharpoons[k_1, k_{-1}]{k_2} ES \rightarrow E + P \]

\[
\frac{d[ES]}{dt} = k_1 [E] [S] - (k_{-1} + k_2) [ES]
\]

Conservation equations: 
\[ [E]_{total} = [E] + [ES] \] and 
\[ [S]_{total} = [S] + [ES] \]

Quasi-steady-state during a time interval

\[ \frac{d[ES]}{dt} \approx 0 \text{ for } t \in [t_1 ; t_2] \]
- **Mass action modelling**

\[
E + S \xrightleftharpoons[k_{-1}]{k_1} ES \rightarrow E + P
\]

\[
d[ES] / dt = k_1 [E] [S] - (k_{-1} + k_2) [ES] = 0 \text{ at steady state}
\]

Conservation equations: \([E]_{\text{total}} = [E] + [ES]\) and \([S]_{\text{total}} = [S] + [ES]\)

- **Henri equation (1903)**
- **Briggs-Haldane equation (1925)**
- **Known as Michaelis-Menten equation**

\[
V = \frac{V_M [S]}{K_M + [S]}
\]

\[
V_M = k_2 [E]_{\text{total}} \quad K_M = \frac{k_{-1} + k_2}{k_1}
\]

**Non – physiological conditions**
- Henri-Michaelis equation developed for the very simple mechanism:
  
  one substrate $S$ – one product $P$.

- Most of the reaction are bimolecular: $A + B \leftrightarrow P + Q$
Mass action law:

\[ A + B \xleftrightarrow{E} P + Q \]

One can write the mass action law for each individual reaction and the conservation equations:

\[
\frac{d[EA]}{dt} = k_A [E] [A] - (k_A + k_B) [EA] \\
\frac{d[EB]}{dt} = \ldots \\
\frac{d[EPQ]}{dt} = \ldots \\
\]

Conservation equations:

\[
[E]_{\text{total}} = [E] + [EA] + [EB] + [EAB] + [EP] + \ldots \\
[A]_{\text{total}} = [A] + [EA] + [EAB] \ldots \ldots [B]_{\text{total}} = \ldots \\
\]

⇒ Dramatically increases the number of differential and algebraic equations.

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Mass action law:

\[ A + B \rightleftharpoons P + Q \]

One can assume the steady state hypothesis:

\[
\frac{d[EA]}{dt} = k_A [E] [A] - (k_{-A} + k_B) [EA] = 0 \quad \frac{d[EB]}{dt} = 0 \quad \frac{d[EQ]}{dt} = 0
\]

Conservation equations:

\[
[E]_{\text{total}} = [E] + [EA] + [EB] + [EAB] + [EP] + \ldots
\]

\[
[A]_{\text{total}} = [A] + [EA] + [EAB] \ldots
\]

and solve the system of algebraic equations:

⇒ Very complex rate equation.
First observation

\[ A + B \rightleftharpoons P + Q \]

When only one substrate (A for instance) is varied (the other B being maintained constant), a hyperbolic variation is often observed which can be fitted by a Michaelis-Henri equation with a more or less constant « apparent \( K_M \) ».

Extension of the Henri-Michaelis equation:

\[
V = \frac{V_f \times \frac{[A]}{K_A} \times \frac{[B]}{K_B}}{\left[1 + \frac{[A]}{K_A}\right] \times \left[1 + \frac{[B]}{K_B}\right]}
\]

(All the parameters \( K_A, K_B \) and \( V_f \) are equal to 1)
**Second observation**

Many reactions are reversible and the cellular concentrations of the products are non-zero.

→ One has to consider a reverse term of the same type for the products with a competitive inhibition for their corresponding substrates:

\[
V = \frac{V_f \times [A] \times [B]}{K_A K_B} \times \frac{-V_b \times [P] \times [Q]}{K_P K_Q} \\
\left[1 + \frac{[A]}{K_A} + \frac{[P]}{K_P}\right] \times \left[1 + \frac{[B]}{K_B} + \frac{[Q]}{K_Q}\right]
\]

Reversibility

\(V_f = \text{maximal rate in the forward reaction}\)

\(V_b = \text{maximal rate in the backward reaction}\)

When the concentration of the products (substrates) are zero, the kinetic is Michaelian for one substrate (product) at fixed concentration of the other.

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The case of 2 substrates – 2 products – The Haldane relationship

\[
A + B \xrightleftharpoons[V_f]{V_b} P + Q
\]

At equilibrium \( V = 0 \)

\[
\begin{align*}
V_f \times \frac{[A_{eq}]}{K_A} \times \frac{[B_{eq}]}{K_B} &= V_b \times \frac{[P_{eq}]}{K_P} \times \frac{[Q_{eq}]}{K_Q} \\
\Rightarrow \frac{[P_{eq}]}{[A_{eq}]} \times \frac{[Q_{eq}]}{[B_{eq}]} &= K_{eq} = \frac{V_f}{V_b} \times \frac{K_P}{K_A} \times \frac{K_Q}{K_B}
\end{align*}
\]

Haldane relationship

\[
K_{eq} = \frac{V_f}{V_b} \times \frac{K_P}{K_A} \times \frac{K_Q}{K_B} \quad (3)
\]

The kinetic parameters are not independent

- \( K_{eq} \) is a thermodynamic constraint, independent of the kinetic mechanism and is usually known.

- In order to take this thermodynamic constraint into account, \( V_b \) for instance can be expressed as a function of the other parameters and replaced in the rate function (2):

\[
V = \frac{V_f \times \frac{[A]}{K_A} \times \frac{[B]}{K_B} - V_b \times \frac{[P]}{K_P} \times \frac{[Q]}{K_Q}}{1 + \frac{[A]}{K_A} + \frac{[P]}{K_P}} \times \left[1 + \frac{[B]}{K_B} + \frac{[Q]}{K_Q}\right] \quad (2)
\]

\( K_{eq} \) = Equilibrium constant of the whole reaction

\( K_A, K_B, \ldots \) : apparent Michaelis constant of A, B, ….for the enzyme.
The case of 2 substrates – 2 products – $K_{eq}$ into account

\[
A + B \xrightleftharpoons[V_f \downarrow V_b \uparrow]{} P + Q
\]

\[
V = \frac{V_f \times \frac{[A]}{K_A} \times \frac{[B]}{K_B} - V_b \times \frac{[P]}{K_P} \times \frac{[Q]}{K_Q}}{\left[1 + \frac{[A]}{K_A} + \frac{[P]}{K_P}\right] \times \left[1 + \frac{[B]}{K_B} + \frac{[Q]}{K_Q}\right]}
\]  

(2)

Haldane relationship

\[
K_{eq} = \frac{V_f}{V_b} \times \frac{K_P}{K_A} \times \frac{K_Q}{K_B}
\]  

(3)

Advantage:
$K_{eq}$ is taken into account. 
(Haldane relationship is satisfied)

Drawback:
The symmetry between substrates and products is lost.

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**RATE EQUATIONS - 10**

The case of 2 substrates – 2 products – Simple Mass action law

\[
A + B \xrightarrow{V_f} P + Q
\]

\[
V = V_f \times \frac{[A]}{K_A} \times \frac{[B]}{K_B} - V_b \times \frac{[P]}{K_P} \times \frac{[Q]}{K_Q}
\]

(2')

When the saturation processes are not taken into account, (2) can be simplified in (2'):

\[
V = V_f' \times [A] \times [B] - V_b' \times [P] \times [Q]
\]

(2')

\[K_{eq}\] (2') can be introduced in (2') like in (4):

\[
V = V_f' \left( [A] \times [B] - \frac{1}{K_{eq}} \times [P] \times [Q] \right)
\]

(4')

**Advantage:**
Very simple equation; can be used in big networks for a preliminary approach

**Drawbacks:**
- Only valid on a limited range of concentrations.
- Non saturable function:
the rate can increase indefinitely as a function of metabolites concentrations.

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- **Competitive inhibition (with the substrate)**

\[
E + S \xrightleftharpoons[k_{-1}]{k_1} ES \rightarrow E + P
\]

\[
K_M \Rightarrow K'_M = K_M \left(1 + \frac{[I]}{K_i}\right)
\]

\[V_M\] remains the same, because high concentrations of substrate can eliminate the inhibitor.

- **Non-competitive inhibition (with the substrate)**

\[
E + S \xrightleftharpoons[k_{-1}]{k_1} ES \rightarrow E + P
\]

\[
V_M \Rightarrow V'_M = V_M \frac{1}{\left(1 + \frac{[I]}{K_i}\right)}
\]

Non-competitive inhibitors are worthwhile as drugs, because they are active whatever the substrate concentration (the substrate accumulates following the reaction inhibition).
SIGMOIDAL CURVES – HILL EQUATION

- Corresponds to the simultaneous binding of $n$ S molecules.

$$
E + n\ S \xrightleftharpoons{K_S} ES^n
$$

$$
V = \frac{V_M [S]^n}{K_S^n + [S]^n}
$$

Correspond to a limit case, biologically impossible –
Typical example: oxygen binding to hemoglobin.

- Sigmoidal inhibition.

$$
(1 + \frac{[I]}{K_I}) \rightarrow (1 + \frac{[I]^n}{K_I})
$$

$\text{Inhibiteur}$

$V$

$n = 4$

$0\ 1\ 2\ 3\ 4\ 5$

$n = 1$

$n = 2$

$n = 4$

$0\ 0.2\ 0.4\ 0.6\ 0.8\ 1\ 1.2$

$0\ 1\ 2\ 3\ 4\ 5$

$0\ 0.2\ 0.4\ 0.6\ 0.8\ 1\ 1.2$

$[\text{Inhibiteur}]$

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THE TOOL BOX

A pencil and an envelop : use the back.

Spread_sheets :
- Excel, Openoffice etc. (Graphique, Solver; Statbox, etc.)

Differential equations :
- Berkeley Madonna at http://www.berkeleymadonna.com/
  - Scilab at http://www.scilab.org/

Multi-Agents Systems
- NetLogo at http://ccl.northwestern.edu/netlogo/
  - netBioDyn at http://netbiodyn.tuxfamily.org/

Cellular automata : NetLogo;

Petri Nets
- StpnPlay at http://dce.felk.cvut.cz/capekj/StpnPlay/
  - SPNP at http://citeseer.ist.psu.edu/ciardo89spnp.html
  - Groupe Francophone sur les réseaux de Petri at
    http://www3.ec-lille.fr/~rdp/