

CHAPTER 4 - HOW CELLS WORK

4.2 The Central Dogma (p. 105) – see figure 4.1 (p.106)

DNA → transcription → RNA → translation → proteins

4.3 DNA Replication (p. 107)

1. Fig 4.2 – Initiation of DNA synthesis requires formation of an RNA primer
2. Fig. 4.3 – Steps of replication of bacterial chromosome

4.4 Transcription (p. 110) – mRNA, tRNA, rRNA

4.5 Translation; Message to Product (p.113)

4.5.1 Genetic Code – Table 4.1 (p. 114)

1. Code is degenerate – more than one codon specifies a particular amino acid
2. Genetic engineering–code universal –human protein understood by E.coli , yeast

4.5.2 – Translation- (1) initiation, (2) elongation, and (3) termination

4.5.3 – Posttranslational Processing -

1. Protein secretion through Cytoplasmic Membrane in prokaryotes –(p. 117)
 - a. Gm -: outer membrane blocks release into extracellular compartment
 - b. Gm +: proteins readily pass through cell wall into extracellular compartment
2. Protein secretion in eucaryotes; 2 pathways by exocytosis. A bioprocess engineer must be aware that many proteins are subject to extensive processing after the initial polypeptide chain is made.

(1) Constitutive exocytosis pathway – operates at all times

(2) Regulated exocytosis pathway – secrete only in response to chemical signals

(3) N-linked glycosylation (addition of sugars) – pattern targets protein to a

compartment or degradation and removal from the organism if not humanlike. Involves both ER and Golgi. (p.117) USE OF PROCARYOTIC CELLS (E. coli) TO SERVE AS HOSTS FOR EXPRESSION OF HUMAN THERAPEUTIC PROTEINS is limited to those proteins where N-linked glycosylation is NOT present or is UNIMPORTANT. Even mammalian cells will show altered patterns of glycosylation when cultured in BIOREACTORS and patterns can shift upon SCALE-UP.

4.6 (p. 119) Metabolic Regulation – HEART OF A LIVING CELL

4.6.1 Genetic-level Control (Which proteins are synthesized?)

1. Transcriptional control – most common strategy. Use Repressor Protein
 - a. Feedback Repression – by end product (Fig. 4.9, p.120)
 - b. Induction – by substrate (Fig. 4.10, p.120).
2. Definition- OPERON – set on contiguous genes, encoding proteins with related functions, under the control of a single promoter-operator.
3. Example- lac operon – controls synthesis of 3 proteins in lactose use as C&E source.
 - (1) lac z – encodes B-galactosidase (lactase); lactose → glucose + galactose
 - (2) lac y – permease – increase rate of uptake of lactose into the cell
 - (3) role of cAMP – increases as energy decreases; cAMP binds to CAP to form a complex that binds near promoter and enhances RNA polymerase binding to lac promoter

4. Regulon – noncontiguous gene products under control of separate promoters can be coordinately expressed in a regulon. (e.g., N&P starvation, aerobic to anaerobic)

1. Constitutive – unregulated genes. Enzymes involved in glycolysis.
2. Example 4.1 – Diauxic Growth (see Figure 4.11) and Explain

4.6.2 – Enzymatic Level Control – Metabolic Pathway Control (p. 123) - Inhibition – occurs at the enzyme level (See Figure 4.12).

Fermentation Specialist – tries to disrupt the cell's control to cause cell to overproduce product of commercial interest.

1. Isozymes - 2 separate enzymes, each sensitive to a different end product.
2. Concerted Feedback Inhibition – 1 enzyme with 2 allosteric binding sites, need high levels of P_1 and P_2 for full inhibition.
3. Sequential Feedback Inhibition - P_1 inhibits E_4 ; P_2 inhibits E_5 . Need both P_1 and P_2 levels to be high to fully inhibit reaction.
4. Cumulative Feedback Inhibition – effector sites for several end products

4.7 (p.124) How the Cell Senses its Extracellular Environment

4.7.1 – Mechanisms to Transport Small Molecules across Cellular Membranes

A. Energy-Independent mechanisms

1. Passive Diffusion (water, oxygen)
2. Facilitated Diffusion (sugars-eucaryotes, glycerol-procaryotes)

B. Energy-Dependent mechanisms

1. Active Transport
2. Group Translocation

A1. Passive Diffusion: Concentration gradient: $J_A = K_p (C_{AE} - C_{AI})$
(4.1)

J = flux across membrane (mol/ cm^2 - s)

K_p = permeability (cm/s); C_{AE} = extracell conc. of species A (mol/ cm^3)

C_{AI} = Intracell conc. of species A (mol/ cm^3)

Hydrophobic compounds – high diffusivities (10^{-8} cm^2/s) in cell membranes

A2. Facilitated Diffusion: protein carrier molecule (embedded in membrane) binds reversibly with target (A), undergoes conformational change to release A on Intracell side of membrane.

$$J_A = J_{A \max} \frac{C_{AE}}{K_{MT} + C_{AE}} - \frac{C_{AI}}{K_{MT} + C_{AI}} \quad (4.2)$$

K_{MT} = binding affinity of the substrate (mol/ cm^3)

$C_{AE} > C_{AI} \rightarrow$ net flux INTO cell; $C_{AE} < C_{AI} \rightarrow$ net flux OUT of cell

B1. Active Transport: AGAINST a concentration gradient; proteins in membrane

Energy Sources: (1) pH gradient of proton-motive force; (2) secondary gradients (e.g., Na^+ , or other ions) derived from proton-motive force by other transport mechanisms and by hydrolysis of ATP.

(1) Proton-motive force: See Figure 5.6, p. 142. Tendency of protons to return to the inside of the membrane.

Hydrogen atoms removed from NADH are carried to outside of membrane.

e^- are returned to the cytoplasmic side of the membrane to combine with O_2 and H^+ the cytoplasm to form OH^- on the inside.

A pH gradient is created across the membrane with OH^- inside and H^+ outside

(2) Molecules transported without coupling to the ion gradients generated by the proton-motive force. Hydrolysis of ATP to release phosphate bond energy is used DIRECTLY in transport (maltose in E. coli).

$$J_A = J_{A \max} \frac{C_{AE}}{K_{MT} + C_{AE}} \quad (4.3)$$

B2. Group Translocation. Chemical modification of substrate during transport.

Example – Phosphotransferase System – uptake of sugars in bacteria, with source of energy = Phosphoenolpyruvate (PEP).



By converting sugar to phosphorylated form, sugar is trapped inside the cell. Preferable to active transport (energy to move sugar into cell, then energy to phosphorylate it).

4.7.2 (p. 127) Role of Cell receptors in Metabolism and Cell Differentiation

Taxis (Surface Receptors) – Response of bacteria involving receptors binding to specific compounds that result in a change in the direction of movement of the flagella.

Examples (chemotaxis, aerotaxis, phototaxis), positively, negatively

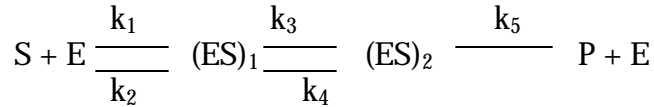
Quorum sensing molecule (Intracellular Receptors) – produced by bacteria, whose accumulation is related to cell concentration (biofilms). e.g., acylated homoserine lactone

Higher cells – differentiation (Surface Receptors) – e.g., Steroids – NOT act by themselves, but the hormone-receptor complex interacts with gene loci to activate transcription of a target gene. E.g., Cell Adhesion can lead to changes in cell morphology that are critical to animal cell growth and physiological function.

Date: 9/16/03

Date Due: 9/26/03 (ABET code from syllabus provided for each problem)

1. (1,3,b,e) Enzyme kinetics. Consider the following enzyme reaction sequence:



Develop a suitable rate expression for product formation [$v = k_5(ES)_2$] by using:

- (a) the equilibrium approach, and
 - (b) the quasi-steady-state approach
2. (1, 3, b, c, e) Solve problem 3.3, page 98 of the textbook
- a. What is the function of fumarase?
3. (1,3,b,c,e) Solve problem 3.6, page 99 of the textbook
4. (1,3,b,c,e) Solve problem 3.9, page 100 of the textbook
5. (1,3,b, c, e) Solve problem 3.16, page 102 of the textbook
- a. What type of inhibition is this?
 - b. Determine the constants V_m , K'_m , and K_I
 - c. Could you modify the operation of a biochemical reactor in order to minimize the effect of the inhibitor? If so, how?
6. (1, 2, 3, b, e) *Serratia marcescens* is cultured in a minimal medium reactor. Oxygen consumption is measured at a cell concentration of 22.7 g/L dry weight.
- | Time (min) | Oxygen Conc. (mmol/L) | Time (min) | Oxygen Conc. (mmol/L) |
|------------|-----------------------|------------|-----------------------|
| 0 | 0.25 | 10 | 0.18 |
| 2 | 0.23 | 12 | 0.16 |
| 5 | 0.21 | 15 | 0.15 |
| 8 | 0.20 | | |
- a. Determine the best kinetic model fit to the data
 - b. Determine the rate constant
7. (1, 2, 3, b, 3) An enzyme is immobilized on a flat sheet of polymer and placed in a stirred reactor. The enzyme intrinsic maximum reaction rate is 6×10^{-6} mol/s-mg enzyme. The amount of enzyme bound to the surface is 1×10^{-4} mg enzyme/cm² of support. The K_m value in solution is 2×10^{-3} mol/L. The mass transfer coefficient is 4.3×10^{-5} . (A) What is the reaction rate when the bulk concentration of substrate is 4×10^{-3} mol/L? (B) What is the substrate surface concentration? (C) What is the Da value for this system?
2. (1, 2, b,e) Describe simple experiments to determine if the uptake of a nutrient is by (a) passive diffusion, (b) facilitated diffusion, (c) active transport, or (d) group translocation.

Membranes - Active Transport Problem:

Given: The concentration of chloride ion in blood serum is about 0.10 M. The concentration of chloride ion in urine is about 0.16 M.

Find: (1) The energy expended by the kidneys in transporting chloride from plasma to urine; and (2) how many moles of Cl⁻ ions could be transported per mole of ATP hydrolyzed?

Solution:

$$\begin{aligned}(1) \quad \Delta G &= 2.3 RT \log \frac{C_2}{C_1} \\ &= 2.3 (1.987 \text{ cal/mol}) (298) \log \frac{0.16}{0.10} \\ &= 1362 \text{ cal/mol} (0.204) \\ &= 278 \text{ cal/mol}\end{aligned}$$

(2) ATP Hydrolysis provides 7700 cal/mole:

$$\frac{7700 \text{ cal/mole ATP}}{278 \text{ cal/mole Cl}^{-1}} \simeq 28 \text{ Cl}^{-1} / \text{ATP}$$

The standard free energy change for the movement of an uncharged molecular from one side of a membrane at concentration C_1 to the other at concentration C_2 under nonequilibrium conditions is:

$$\Delta G = 2.3 RT \log \frac{C_2}{C_1}$$

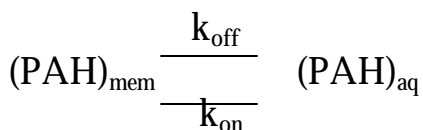
Transfer of Polycyclic Aromatic Hydrocarbons Between Model Membranes: Relation to Carcinogenicity.

Plant, A.L., H.J. Pownall, and L. C. Smith.

Chem.-Biol. Interactions, 44: 237-246 (1983)

Key Concepts and Questions:

Metabolism of PAH by cytochrome P-450 is a prerequisite to carcinogenic activity.



Molecular Volume of the PAH is a rate-determining factor.

Donor vesicles (POPC with PAH) and Acceptor vesicles (POPC without PAH)

Question: Rate limiting step is?

Thermodynamic parameters determined from a van't Hoff Arrhenius plot (Fig. 3)

E_a , ΔG , ΔH , ΔS – Table 1 (p.242)

Question: Calculate the E_a value from Fig. 3.

Question: Endoplasmic reticulum – what is its role?

p. 240. Solvation of a hydrophobic molecule ... requires reorganization of water structure. Size of cavity formed to accommodate the hydrocarbon molecule is proportional to its molecular area.

p.243. Rate of transfer of lipophilic materials out of membranes reflects equilibrium partitioning. If active site of P-450 is within the hydrophobic environment of the membrane, characteristics of partitioning will determine extent of enzyme-substrate complex formation.

Dissociation constant for B(a)P from microsomes = 1 micromolar

K_m for hydroxylation of B(a)P by microsomes \approx 1 micromolar

Question: What is the likely mechanism for the transfer/transport of PAH between membrane and aqueous phase? Explain your answer.