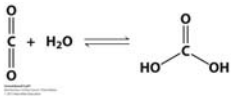
Basic Concepts of Enzyme Action

Stryer Short Course
Chapter 6

Enzymes

- Biocatalysts
- Active site
- Substrate and product
- Catalyzed rate
- Uncatalyzed rate



Rate Enhancement

Table 6.1 Rate enhancement by selected enzymes

Enzyme	Nonenzymatic half-life	Uncatalyzed rate (k_{un} s^{-1})	Catalyzed rate (k_{cat} s^{-1})	Rate enhancement (k_{cat} s^{-1}/k_{un} s^{-1})
OMP decarboxylase	78,000,000 years	2.8×10^{-16}	39	1.4×10^{17}
Staphylococcal nuclease	130,000 years	1.7×10^{-13}	95	5.6×10^{14}
AMP nucleosidase	69,000 years	1.0×10^{-11}	60	6.0×10^{12}
Carboxypeptidase A	7.3 years	3.0×10^{-9}	578	1.9×10^{11}
Ketosteroid isomerase	7 weeks	1.7×10^{-7}	66,000	3.9×10^{11}
Triose phosphate isomerase	1.9 days	4.3×10^{-6}	4,300	1.0×10^{9}
Chorismate mutase	7.4 hours	2.6×10^{-5}	50	1.9×10^{6}
Carbonic anhydrase	5 seconds	1.3×10^{-1}	1×10^{6}	7.7×10^{6}

Abbreviations: OMP, orotidine monophosphate; AMP, adenosine monophosphate.
Source: Data from A.Radzicka and R.Wolfenden, Science 267:90–93, 1995.
Table 6.1
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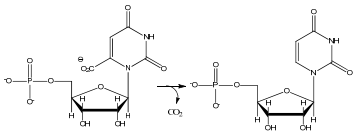
Which is a better catalyst, carbonic anhydride
Or OMP decarboxylase? Defend your answer.

Orotidine Decarboxylase

- Key enzyme in production of nucleotides for DNA
- $T_{1/2}$ = 14 ms
- But what makes it a great enzyme?



The Speed of the Uncatalyzed Rxn

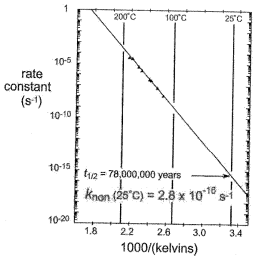
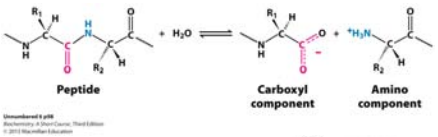
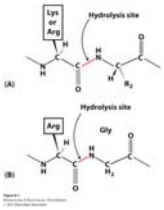


Figure 4 Temperature dependence of the rate of decarboxylation of 1-methyloro-tate in 0.1 M potassium phosphate buffer, pH 6.8 (6).

Substrate Specificity

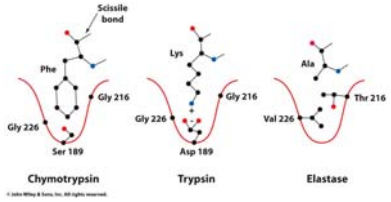


- Example: Proteolytic enzymes
- Trypsin vs. Thrombin



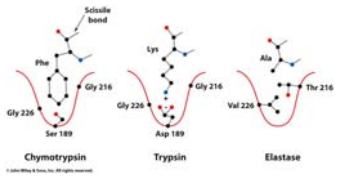
Substrate Specificity

- Specificity pocket
- Binding affinity
- Promiscuity



Question

- What effects would the following mutations have on enzyme specificity?
  - Trypsin D189E
  - Elastin V226A, T216A
  - Trypsin D189K



EC Nomenclature

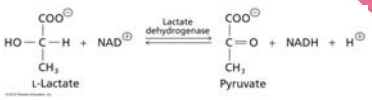
[TABLE 6-2]

Enzyme Classification

Class of Enzyme	Type of Reaction Catalyzed
1. Oxidoreductases	Oxidation–reduction reactions
2. Transferases	Transfer of functional groups
3. Hydrolases	Hydrolysis reactions
4. Lyases	Group elimination to form double bonds
5. Isomerases	Isomerization reactions
6. Ligases	Bond formation coupled with ATP hydrolysis

Enzyme Classes

1. Oxidoreductase



- Recognize Redox reactions
- Redox cofactors: NAD^+/NADH, FAD/$FADH_2$, Q/QH_2
- Dehydrogenases, oxidases, peroxidases, reductase

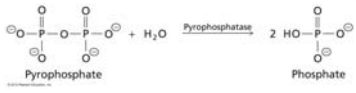
Enzyme Classes

2. Transferase

- 2 substrates
- Coenzymes often involved
- Transferase, kinase

Enzyme Classes

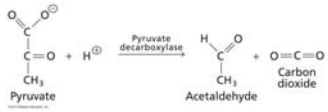
3. Hydrolase



- Water nucleophile
- Phosphatase, nuclease, protease, peptidase

Enzyme Classes

4. Lyase



- Hardest to recognize—not redox, hydrolysis
- Elimination of a group to give double bond
- Reversible
- Hydratase, decarboxylase, (formerly synthases)

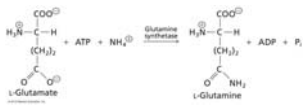
Enzyme Classes

5. Isomerase

- Rearragement without loss/add
- Racemase, isomerase, mutase (phosphate)

Enzyme Classes

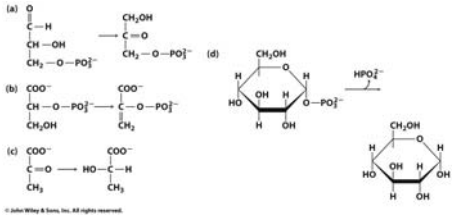
6. Ligase



- Joining together with ATP input
- Irreversible
- Synthetase, carboxylase

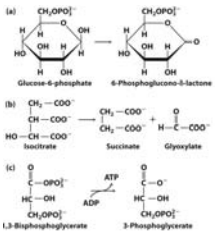
Problem

- To which class do the enzymes that catalyze the following reactions belong?



Problem

- Propose a name for each enzyme.



Cofactors

- Apoenzyme vs holoenzyme
- Cofactors
  - Coenzymes
    - Prosthetic groups
    - Cosubstrates
  - Metals
- Function to enable specific chemistry

Table 6.2 Enzyme cofactors

Cofactor	Enzyme*
Coenzyme†	
Thiamine pyrophosphate (TPP)	Pyruvate dehydrogenase
Flavin adenine dinucleotide (FAD)	Monoamine oxidase
Nicotinamide adenine dinucleotide (NAD^+)	Lactate dehydrogenase
Pyridoxal phosphate (PLP)	Glycogen phosphorylase
Coenzyme A (CoA)	Acetyl CoA carboxylase
Biotin	Pyruvate carboxylase
6′-Deoxyadenosyl cobalamin	Methylmalonyl mutase
Tetrahydrofolate	Thymidylate synthase
Metal	
Zn^{2+}	Carbonic anhydrase
Mg^{2+}	*Eco*RV
Ni^{2+}	Urease
Mo	Nitrogenase
Se	Glutathione peroxidase
Mn^{2+}	Superoxide dismutase
K^+	Acetoacetyl CoA thiolase

*The enzymes listed are examples of enzymes that employ the indicated cofactor.
†Often derived from vitamins, coenzymes can be either tightly or loosely bound to the enzyme.

Table 6.2
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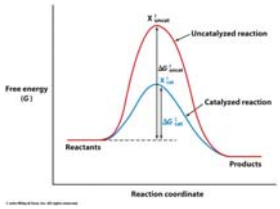
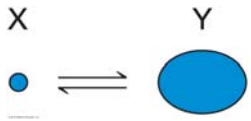
Thermodynamics vs Kinetics

- Gibbs Free Energy
  - Spontaneous
  - Favorable
  - exergonic
- $\Delta G = G_{pdt} - G_{rxt}$
  - Path independent
  - Doesn't tell us about kinetics



Equilibrium

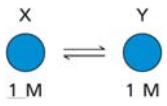
- You can't understand thermodynamics until we clear up some common misconceptions about equilibrium…



- Is this reaction at equilibrium or not?
- If not, in which direction does the equilibrium lie?

Standard Free Energy

- Every reaction moves spontaneously toward **equilibrium—but that could be either direction**
- There is a relationship between equilibrium constant and free energy of the reaction
- If we start with 1M reactants and products, the free energy change of that reaction is called the "standard" free energy
- $\Delta G^{o\prime}$ is a reflection of the chemical potential (stability of bonds)
  - Negative $\Delta G^{o\prime}$ means equilibrium favors pdts
  - Larger $\Delta G^{o\prime}$ means it is favored to a greater degree



- **$\Delta G^{0\prime} = -RT \ln K_{eq}$**
- The ^{0} means "standard"
  - 1 M, 1 atm, 298 K
- The ' means "biological standard"
  - pH 7, 55M water

Standard Free Energy

Glutamate + NH_4^+ ⇌ glutamine + H_2O

$\Delta G^{\circ\prime}_{reaction} = +14$ kJ mol^{-1}

[TABLE 12-4]

Standard Free Energy Change for Phosphate Hydrolysis

Compound	$\Delta G^{\circ\prime}$ (kJ · mol^{-1})
Phosphoenolpyruvate	−61.9
1,3-Bisphosphoglycerate	−49.4
ATP → AMP + PP_i	−45.6
Phosphocreatine	−43.1
ATP → ADP + P_i	−30.5
Glucose-1-phosphate	−20.9
PP_i → 2 P_i	−19.2
Glucose-6-phosphate	−13.8
Glycerol-3-phosphate	−9.2

- What do these examples mean?
  - Under standard conditions, glutamine will spontaneously turn into glutamate.
  - Hydrolysis of ATP is more favorable than hydrolysis of glucose-6-phosphate

Standard Free Energy vs. Free Energy

ATP ⇌ ADP + P_i $\Delta G^{o\prime}$ = -32 kJ and ΔG = -32 kJ

1 M 1 M

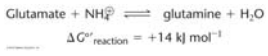
ATP ⇌ ADP P_i $\Delta G^{o\prime}$ is -32 kJ ΔG = zero

Quantitative Problems

- What is [product]/[rxt] ratio of ATP hydrolysis to ADP at equilibrium?
  - **$\Delta G^{0\prime} = -RT \ln K_{eq}$**
  - R = 8.314 J/mol K, T in Kelvin
  - [ADP][Pi]/[ATP] = $4.1 \times 10^5 = K_{eq}$
- What is the free energy of ATP hydrolysis when it reaches equilibrium?
  - Equilibrium = DEAD!

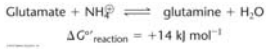
A Second Misconception…

- I have mixed together some glutamate, ammonia, glutamine, and water. Will my reaction proceed spontaneously to the left or to the right?



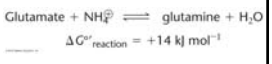
A Second Misconception…

- We don't know—it depends on HOW MUCH of each you have mixed together.
- Reactions always move spontaneously toward equilibrium, but we need to know ACTUAL CONCENTRATIONS to determine which direction that is



Example

- Standard Free energy allows us to calculate equilibrium concentrations
- K_{eq} = 0.00352, so for example
  - [glutamine] = 1 mM
  - [NH_4^+] = 0.53M
  - [glutamate] = 0.53M



- Fill in the table

[glutamine]	[NH_4^+]	[glutamate]	Right or left?
1 M	1 M	1 M	
0.1 mM	0.53M	0.53M	
1 mM	0.53M	0.23M	

Free Energy

- The free energy of a PARTICULAR reaction depends on two factors
  - The nature of the bonds in the reaction
  - The concentration of the compounds
- A reaction with a $-\Delta G^{o\prime}$ can be spontaneous or nonspontaneous under cellular conditions.

$$\Delta G = \Delta G^{0\prime} + RT \ln \frac{[pdts]}{[rxts]}$$

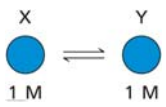
Free Energy of ATP hydrolysis

- Actual cellular concentrations don't vary much from $[P_i]$=[ATP] = 5 mmol and [ADP]= 1 mmol
- Problem: What is the actual free energy of ATP hydrolysis in the cell? More or less than -32 kJ? What does this mean, physiologically?

$$\Delta G_{reaction} = \Delta G^{\circ\prime}_{reaction} + RT \ln \frac{[products]}{[reactants]}$$

Standard Free Energy vs. Free Energy

$\Delta G = \Delta G^{0\prime} + RT \ln \frac{[pdts]}{[rxts]}$

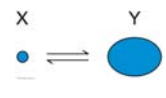


In this example, $\Delta G^{o\prime}$ is negative, so ΔG is negative because $\Delta G = \Delta G^{o\prime}$.

$\Delta G = \Delta G^{0\prime} + RT \ln \frac{[pdts]}{[rxts]}$

$0 = \Delta G^{0\prime} + RT \ln K_{eq}$

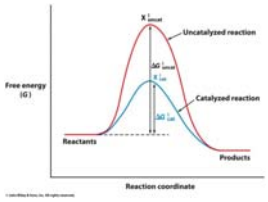
$\Delta G^{0\prime} = -RT \ln K_{eq}$



Once it reaches equilibrium, $\Delta G^{o\prime}$ is still the same value, but ΔG has reached zero.

Energy Diagrams

- Energy Diagrams are a measure of the start of a reaction (often standard conditions)
- The free energy changes as the reaction progresses because the concentrations change

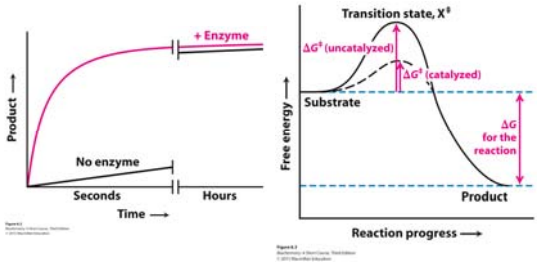


Rule of Thumb

Table 6.3 Relation between $\Delta G'^{\circ}$ and K'_{eq} (at 25°C)

K'_{eq}	$\Delta G'^{\circ}$ kJ mol^{-1}	$\Delta G'^{\circ}$ kcal mol^{-1}
10^{-5}	28.53	6.82
10^{-4}	22.84	5.46
10^{-3}	17.11	4.09
10^{-2}	11.42	2.73
10^{-1}	5.69	1.36
1	0	0
10	−5.69	−1.36
10^{2}	−11.42	−2.73
10^{3}	−17.11	−4.09
10^{4}	−22.84	−5.46
10^{5}	−28.53	−6.82

Kinetics

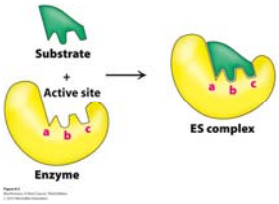


How Enzymes Change Kinetics

- Two major effects on mechanisms—any or all may be used in a given enzyme
  - Chemical Mechanisms (later chapters)
    - Acid-base catalysis
    - Covalent catalysis
    - Metal ion catalysis
  - Binding Mechanisms (this chapter)
    - Proximity/orientation effect
    - Transition State Stabilization

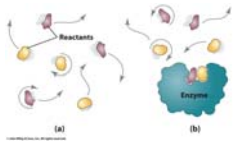
Binding Energy

- Binding based on intermolecular forces
- "Lock and Key"
- Rate Enhancement
  - Orientation
  - Effective concentration



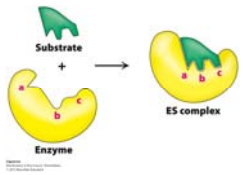
Orientation

- Productive orientation of two molecules in the active site
- "Entropy trap"



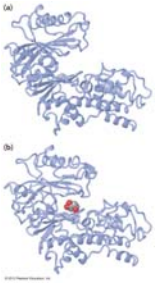
Induced Fit

- "Lock and Key" too simplistic
- Enzymes are actually somewhat flexible
- Substrate specificity comes at catalytic price
- Lower rate, but worth cost



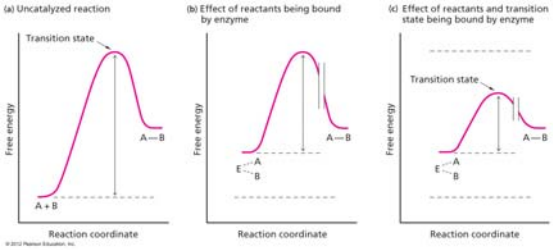
Induced Fit

- Example: hexokinase
- Two loops apart until glucose binds
- Then ATP→ ADP
- If site were closed, then water could enter and ATP→ADP without glucose
- Net hydrolysis of ATP with no purpose



Glucose + ATP ⟹ Glucose 6-phosphate + ADP

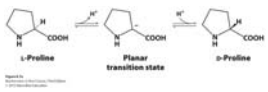
Lowering Activation Energy

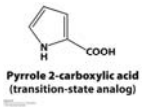


- Enzyme binds TS tighter than starting material

Transition State Analogs

- Can serve as inhibitors
- Example: Proline racemase
  - Which class?
- Error in figure
  - TS vs high energy intermediate





Weak Binding of Substrate

- TS binding stabilitzation is only half of the story
- Substrate binding: can have too much of a good thing
- Thermodynamic pit
- Substrate half bound ~ 10^{-4} M