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Biochemistry: A Short Course

Second Edition

CHAPTER 6

Basic Concepts of Enzyme Action

Chapter 6 Opener
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The activity of an enzyme is responsible for the glow of the luminescent jellyfish. The enzyme **aequorin** catalyzes the oxidation of a compound by oxygen in the presence of calcium to release CO_2 and light.

Chapter 6 Outline

6.1 Enzymes Are Powerful and Highly Specific Catalysts

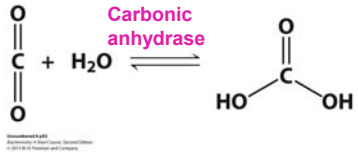
Enzymes **accelerate reactions** by factors of as much as **a million** or more

Table 6.1 Rate enhancement by selected enzymes

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

Abbreviations: OMP, orotidine monophosphate; AMP, adenosine monophosphate.
Source: After A. Radzicka and R. Wolfenden, *Science* 267:90-93, 1995.

Most reactions in biological systems are catalyzed by enzymes



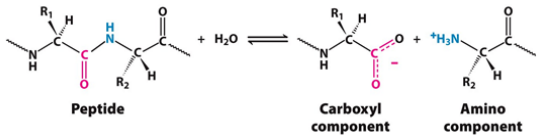
- Carbonic anhydrase is one of the fastest enzymes known
- Each enzyme molecule can hydrate 10^6 molecules of CO_2 /*s*. The catalyzed reaction is 10^7 times as fast as the uncatalyzed one.
- This reaction facilitates the transport of **CO_2** from the **tissues** (where it is produced) to the **blood** and then to the **lungs** (where it is exhaled). The transfer of CO_2 would be less complete in the absence of this enzyme.

Enzymes are **highly specific**

- Enzymes are **highly specific** both in the **reactions** that they catalyze and in their choice of **reactants**, which are called ***substrates***.
- An enzyme usually catalyzes **a single chemical reaction** or a set of closely related reactions.

Proteolytic Enzymes and Enzyme Specificity

- **Proteolytic enzymes** catalyze the **hydrolysis of a peptide bond**

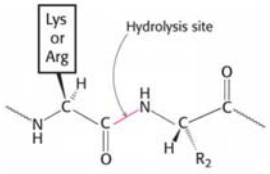


- The hydrolysis of a peptide bond is **thermodynamically favorable** (spontaneous reaction that proceeds without input of energy), but it is **very slow** in the absence of enzymatic catalysis (the lifetime of a peptide bond in aqueous solution in the absence of a catalyst approaches 1000 years-Chapter 4)

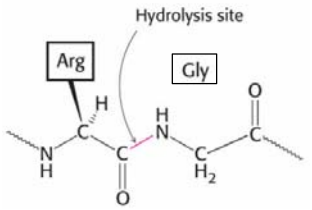
Proteolytic Enzymes

Proteolytic enzymes differ markedly in their degree of substrate specificity

- The **specificity** of an enzyme is due to the **precise interaction** of the substrate with the enzyme. This precision is a result of the **three-dimensional structure of the enzyme**
- **Papain** (found in papaya plants) will cleave any peptide bond with little regard to the identity of the adjacent side chains. This lack of specificity accounts for its use in meat-tenderizing sauces.
- **Trypsin**, a digestive enzyme, is specific and catalyzes the hydrolysis of peptide bonds only on the **carboxyl side** of **lysine** and **arginine** residues



- **Thrombin**, an enzyme that participates in blood clotting, is even more specific than trypsin. It catalyzes the hydrolysis of **Arg–Gly** ***bonds in particular peptide sequences only***



There Are Six Major Classes of Enzymes

1. **Oxidoreductase** catalyze oxidation-reduction reactions.
2. **Transferases** move functional groups between molecules.
3. **Hydrolyases** cleave bonds with the addition of water.
4. **Lyases** remove atoms to form double bonds or add atoms to double bonds.
5. **Isomerases** move functional groups within a molecule.
6. **Ligases** join two molecules at the expense of ATP.

EC (Enzyme Commission) Nomenclature

- The six groups (classes) of enzymes were subdivided so that a four-digit number preceded by the letters *EC*
- Example:
  1. Trypsin cleaves bonds by the addition of water: member of group 3 **Hydrolyases**
  2. Trypsin cleaves only peptide bonds: 3.4.
  3. Trypsin employs a **serine residue** to facilitate hydrolysis and **cleaves the protein chain internally** (in contrast with the removal of amino acids from the end of the polypeptide chain) ⇒ sub-sub-group 21 and identified as 3.4.21.
  4. Trypsin cleaves peptide bonds in which the amino acid donating the carboxyl group to the peptide bond is either **lysine** or **arginine**. Thus, the number uniquely identifying trypsin is EC 3.4.21.4.

6.2 Many Enzymes Require Cofactors for Activity

- The catalytic activity of many enzymes depends on the presence of small molecules: **cofactors**.
- The precise role varies with the cofactor and the enzyme.
- **Apoenzyme**: an enzyme without its cofactor;
- **Holoenzyme** the complete, catalytically active enzyme

- **Cofactors** can be subdivided into two groups (Table 6.2):

(1) Coenzymes: small organic molecules, derived from vitamins
  - **Tightly bound** coenzymes are called **prosthetic (helper) groups.**
  - **Loosely associated** coenzymes are more like **cosubstrates** because, like substrates and products, they **bind** to the enzyme and **are released** from it.
    - Coenzymes are distinct from normal substrates not only because they are often derived from vitamins but also because they are used by a variety of enzymes. Different enzymes that use the same coenzyme usually carry out similar chemical transformations.

(2) Metals

Table 6.2 Enzyme cofactors

Cofactor	Enzyme*
Coenzyme†	
Thiamine pyrophosphate (TPP)	Pyruvate dehydrogenase
Flavin adenine nucleotide (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+-3+}	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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6.3 Free Energy Is a Useful Thermodynamic Function for Understanding Enzymes

- Enzymes speed up the **rate** of chemical reactions (**kinetics**) but do not change the reaction **equilibrium** (**thermodynamics**)
- Whether the reaction can take place **spontaneously** (without an input in energy) depends on the **free-energy difference (Δ*G*)** between the products and the reactants (**$\Delta G = G_{Products} - G_{Reactants}$**)
- ***Free energy* (*G*)** is a thermodynamic property that is a measure of useful energy, or energy that is capable of doing work that can be extracted from a system.

The Free-Energy Change, ΔG

1. A reaction is **spontaneous** only if **ΔG is negative (exergonic reactions).**
   - A spontaneous reaction will take place without the input of energy and, the reaction releases energy.
2. A reaction is at equilibrium when **ΔG = 0.** In a system at equilibrium, there is no net change in the concentrations of the products and reactants
3. A reaction is **nonspontaneous** if **ΔG is positive (endergonic reactions).**
   - A nonspontaneous reaction will take place only with an input of energy.
4. The **ΔG** of a reaction is **independent of the path** (molecular mechanism) of the reaction. The ΔG of a reaction depends only on the free energy difference between products and reactants (**$\Delta G = G_{Products} - G_{Reactants}$**).
   - *The mechanism of a reaction has no effect on ΔG*. For example, the ΔG for the transformation of glucose into CO_2 and H_2O is the same whether it takes place by combustion or by a series of enzyme-catalyzed steps in a cell.
5. The ΔG provides **no information about the rate of a reaction**. The rate of a **reaction depends** on the **free energy** of **activation (ΔG‡)**, which is largely unrelated to the ΔG of the reaction.

The Standard Free-Energy Change of a Reaction Is Related to the Equilibrium Constant

For the reaction: $A + B \rightleftharpoons C + D$

•Standard State

- **ΔG°** is the ***standard free-energy change,*** the free-energy change for this reaction under **standard conditions** (temperature **298 K**; partial pressure of each gas **1 atm**; concentration of each solute **1 M**); *R* is the gas constant, *T* is the temperature (in Kelvin) ⇒ **ΔG°** is **a constant** for a given reaction

•Biochemical standard state

- Because **biochemical systems** commonly involve H^+ concentrations far below 1 M, biochemists define a **biochemical standard free-energy change ($\Delta G^{\circ\prime}$)**, at **$[H^+] = 10^{-7}$ M** (pH 7) and **$[H_2O] = 55.5$ M**.
- For reactions that involve Mg^{2+} (which include most of those with ATP as a reactant), $[Mg^{2+}]$ in solution is commonly taken to be constant $[Mg^{2+}] = 1$ mM.
- Physical constants based on this **biochemical standard state** are called **standard *transformed* constants** and are written with a prime (**$\Delta G^{\circ\prime}$**; **K'_{eq}**) to distinguish them from the *untransformed constants* used by chemists and physicists.

The Standard Free-Energy Change of a Reaction Is Related to the Equilibrium Constant

For the reaction: $A + B \rightleftharpoons C + D$

$$\Delta G = RT \ln \frac{Q}{K} = RT \ln Q - RT \ln K$$

$$Q = \frac{[C]\cdot[D]}{[A]\cdot[B]} \qquad K = \frac{[C]_{eq}\cdot[D]_{eq}}{[A]_{eq}\cdot[B]_{eq}}$$

- ***Q* = Reaction quotient**; the concentrations of reactants and products are not measured at equilibrium
- ***K* = Equilibrium constant**; the concentrations of reactants and products are measured at equilibrium

In standard state all concentrations are 1M ⇒ Q = 1 ⇒ ln Q = 0 and **ΔG *becomes* ΔG°** ⇒ **$\Delta G^\circ = -RT \ln K$**

Thus $\Delta G = \Delta G^\circ + RT \ln \frac{[C][D]}{[A][B]}$

$\Delta G^{\circ\prime}$ and K'_{eq}

- The equilibrium constant under biochemical standard conditions, is **K'_{eq}**

$$\Delta G^{\circ\prime} = -RT \ln K'_{eq} \quad \text{or} \quad K'_{eq} = e^{-\Delta G^{\circ\prime}/RT}$$

- Substituting $R = 8.314 \times 10^{-3}$ kJ mol^{-1} K^{-1} and $T = 298$ K gives

$$K'_{eq} = e^{-\Delta G^{\circ\prime}/2.47}$$

- It is important to stress that whether the **ΔG** for a reaction is larger, smaller, or the same as **$\Delta G^{\circ\prime}$** depends on the concentrations of the reactants and products (Q) at that moment in the reaction.

$$\Delta G = \Delta G^{\circ\prime} + RT \ln \frac{[C][D]}{[A][B]}$$

- **The criteria for spontaneity of a reaction is ΔG, not $\Delta G^{\circ\prime}$**
- This point is important because reactions that are not spontaneous, can be made spontaneous by adjusting the concentrations of reactants and products. This principle is the basis of the coupling of reactions to form metabolic pathways (Chapter 15).

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

K'_{eq}	$\Delta G^{\circ\prime}$ kJ mol^{-1}	$\Delta G^{\circ\prime}$ 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

Enzymes Alter the Reaction Rate but Not the Reaction Equilibrium

- Enzymes **do not alter the equilibrium of a chemical reaction**. The equilibrium position is a function only of the free-energy difference between reactants and products.

Substrate ⇌ Product

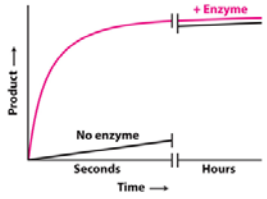


Figure 6.2 Enzymes accelerate the reaction rate. The amount of product formed is the same in the presence and absence of enzyme; the time it takes to reach the product concentration is different (seconds vs. hours)

- Why does the rate of product formation level off with time? The reaction has **reached equilibrium**.

6.4 Enzymes Facilitate the Formation of the Transition State

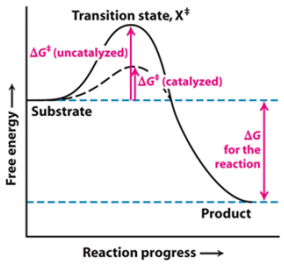
- A chemical reaction of substrate S to form product P goes through a ***transition state*** **X‡** that has a higher free energy than does either S or P.

$$S \rightleftharpoons X^{\ddagger} \rightarrow P$$

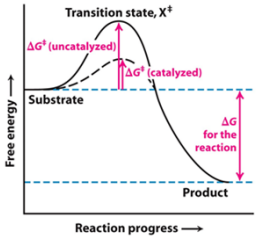
- The transition state is the **least-stable** and most-seldom-occurring species along the reaction pathway because it is the one with the highest free energy

$$\Delta G^{\ddagger} = G_{X}{}^{\ddagger} - G_{S}$$

- The difference in free energy between the transition state and the substrate is called the ***free energy of activation*** or simply the ***activation energy***, symbolized by $\Delta G^{\ddagger}$



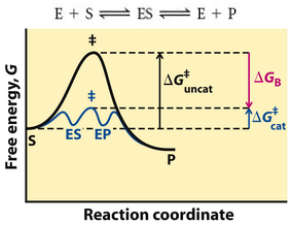
The essence of catalysis is stabilization of the transition state



- Enzymes accelerate reactions by **decreasing ΔG‡, the free energy of activation**, thus *facilitating the formation of the transition state*.
- The combination of substrate and enzyme creates **a reaction mechanism whose transition-state energy is lower than what it would be without the enzyme**

- Because the activation energy is lower, more molecules have the energy required to reach the transition state and more product will be formed faster.

The Binding Energy Between Enzyme and Substrate Is Important for Catalysis



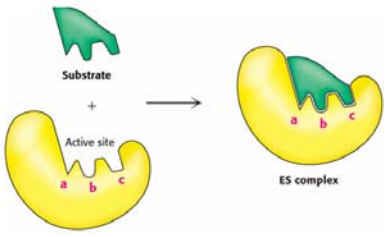
- some weak interactions are formed in the ES complex
- the full complement of such interactions between substrate and enzyme is formed only when the substrate reaches the transition state.
- the transition state is not a stable species but a brief point in time that the substrate spends atop an energy hill

Role of binding energy in catalysis. When the enzyme binds the substrate in the transition state, energy is released: **binding energy (ΔG_B)**. This allows for the lowering of the activation energy for the enzyme catalyzed reaction, compared with the noncatalyzed reaction. **Binding energy is** contributed by formation of weak noncovalent interactions between substrate and enzyme in the transition state. *Lehninger, Principles of Biochemistry*

The Formation of an Enzyme–Substrate Complex Is the First Step in Enzymatic Catalysis

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

- Most enzymes are highly selective in the substrates that they bind
- The substrate or substrates are bound to a specific region of the enzyme called the ***active site***. The active site is the region of the enzyme that most directly lowers the ΔG‡ of the reaction



The Active Sites of Enzymes Have Common Features

1. The active site is a 3-D cleft or crevice

•Lysozyme, found in a variety of organisms and tissues including human tears, degrades the cell walls of some bacteria.

•In **lysozyme**, the important groups in the active site are contributed by residues numbered **35**, **52**, **62**, **63**, **101**, and **108** in the sequence of 129 amino acids (Figure 6.4).

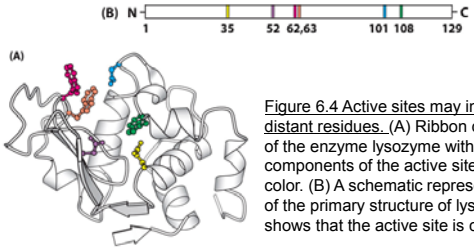


Figure 6.4 Active sites may include distant residues. (A) Ribbon diagram of the enzyme lysozyme with several components of the active site shown in color. (B) A schematic representation of the primary structure of lysozyme shows that the active site is composed

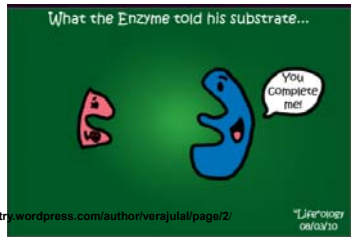
2. The active site takes up a small part of the total volume of an enzyme. Most of the amino acid residues in an enzyme are not in contact with the substrate, they serve as a scaffold to create the three-dimensional active site. In many proteins, the remaining amino acids also constitute regulatory sites, sites of interaction with other proteins, or channels to bring the substrates to the active sites.

Binding of a substrate to an enzyme at the active site. The enzyme chymotrypsin, with bound substrate in red (PDB ID 7GCH). Some key active-site amino acid residues appear as a red splotch on the enzyme surface. *Lehninger, Principles of Biochemistry*

3. Active sites are unique microenvironments. The close association between the active site and the substrate means that **water is usually excluded** from the active site unless it is a reactant. The nonpolar micro-environment of the cleft enhances the binding of substrates as well as catalysis.

4. Substrates are bound to enzymes by multiple weak attractions. The noncovalent, weak, interactions between the enzyme and the substrate in ES complexes are reversible: **electrostatic interactions, hydrogen bonds, van der Waals interactions, hydrophobic interactions**.

•Because the enzyme and the substrate interact by means of short-range forces that require close contact, and because Van der Waals forces become significant in binding only when numerous such interactions form ⇒ **to bind as strongly as possible, the enzyme and substrate should have complementary shapes.**



http://artofbiochemistry.wordpress.com/author/verajulal/page/2/

5. There are **2 models** for how the substrate fits into the enzyme active site.
The **lock and key model** (Emil Fischer, 1890). In this model, the **active site** of the **unbound enzyme** is **complementary** in **shape** to the **substrate.**

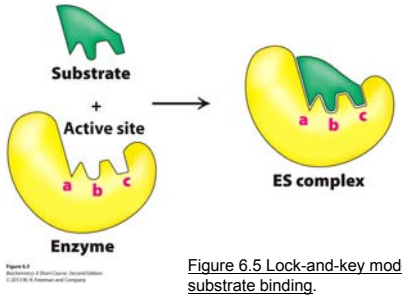


Figure 6.5 Lock-and-key model of enzyme–substrate binding.

The **induced fit model** (Daniel E. Koshland, Jr, 1958). In this model, the **active site** of an enzyme **assumes** a **shape** that is **complementary** to that of the **substrate** only **after** the **substrate** has been **bound** (enzymes are flexible)

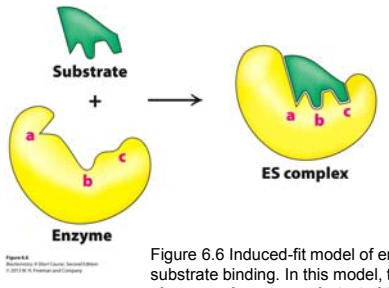
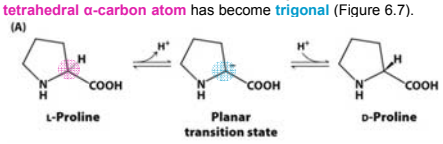


Figure 6.6 Induced-fit model of enzyme–substrate binding. In this model, the **enzyme changes shape on substrate binding.**

Transition-State Analogs Are Potent Inhibitors of Enzymes

Compounds that resemble the transition state of a reaction but are not capable of being acted on by the enzyme are called **transition-state analogs.**
Example: the inhibition of **proline racemase**

- The racemization of proline proceeds through a transition state in which the **tetrahedral α-carbon atom** has become **trigonal** (Figure 6.7).



- The inhibitor pyrrole 2-carboxylate binds to the racemase 160 times as tightly as does proline. **The α-carbon atom of this inhibitor, like that of the transition state, is trigonal.**

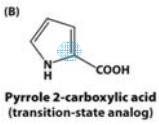


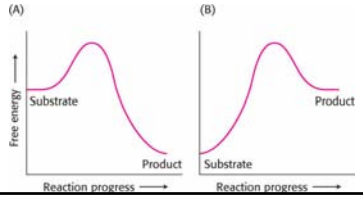
Figure 6.7 Inhibition by transition-state analogs. (A) The isomerization of L-proline to D-proline by proline racemase (B) Pyrrole 2-carboxylate, a transition-state analog because of its trigonal geometry, is a potent inhibitor of proline racemase.

End of Chapter Problems

12. *Give with one hand, take with the other*. Why does the activation energy of a reaction not appear in the final ΔG of the reaction?

Answer: The **ΔG** of a reaction is **independent of the path** (molecular mechanism) of the reaction. The ΔG of a reaction depends only on the free energy difference between products and reactants (**ΔG = $G_{products}$ – $G_{Reactants}$**).

13. *Making progress*. The illustrations below show the reaction-progress curves for two different reactions.



Indicate the activation energy as well as the Δ*G* for each reaction. Which reaction is endergonic? Exergonic?

14. *Mountain climbing*. Proteins are thermodynamically unstable. The Δ*G* of the hydrolysis of proteins is quite negative, yet proteins can be quite stable. Explain this apparent paradox. What does it tell you about protein synthesis?

17. *Match'em*. Match the values K'_{eq} with the appropriate $\Delta G^{\circ\prime}$ values

	K'_{eq}	$\Delta G^{\circ\prime}$ (kJ mol^{-1})
(a)	1	28.53
(b)	10^{-5}	−11.42
(c)	10^{4}	5.69
(d)	10^{2}	0
(e)	10^{-1}	−22.84

$$K'_{eq} = e^{-\Delta G^{\circ\prime}/2.47}$$

18. *Free energy!* Consider the following reaction:
Glucose 1-phosphate ⇌ glucose 6-phosphate
After the reactants and products were mixed and allowed to reach equilibrium at 25° C, the concentration of each compound was measured:
$[\text{Glucose 1-phosphate}]_{eq} = 0.01$ M
$[\text{Glucose 6-phosphate}]_{eq} = 0.19$ M
Calculate K'_{eq} and $\Delta G^{\circ\prime}$

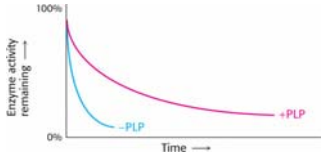
$K'_{eq} = ?$ $\qquad \Delta G^{\circ\prime} = -RT \ln K'_{eq}$

19. *More free energy!* The isomerization of dihydroxyacetone phosphate (DHAP) to glyceroldehyde 3-phosphate (GAP) has an equilibrium constant of 0.0475 under standard conditions (298 K, pH 7).

a) Calculate $\Delta G^{\circ\prime}$ for the isomerization.
b) Calculate ΔG for this reaction when the initial concentration of DHAP is 2×10^{-4} M and the initial concentration of GAP is 3×10^{-6} M. What do these values tell you about the importance of ΔG compared with that of $\Delta G^{\circ\prime}$ in understanding the thermodynamics of intracellular reactions?

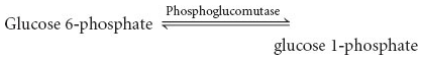
$\Delta G^{\circ\prime} = -RT \ln K'_{eq}$ $\qquad \Delta G = \Delta G^{\circ} + RT \ln \dfrac{[C][D]}{[A][B]}$

21. *A question of stability*. Pyridoxal phosphate (PLP) is a coenzyme for the enzyme ornithine aminotransferase. The enzyme was purified from cells grown in PLP-deficient medium as well as from cells grown in medium that contained pyridoxal phosphate. The stability of the enzyme was then measured by incubating the enzyme at 37° C and assaying for the amount of enzyme activity remaining. The following results were obtained.



(a) Why does the amount of active enzyme decrease with the time of incubation?
(b) Why does the amount of enzyme from the PLP-deficient cells decline more rapidly?

22. *Modified*. Assume that you have a solution of 0.1 M glucose 6-phosphate and 0.01M glucose 1-phosphate. To this solution, you add the enzyme phosphoglucomutase, which catalyzes the reaction.



The $\Delta G^{\circ\prime}$ for the reaction is +7.5 kJ mol^{-1} (+1.8 kcal mol^{-1}).
(a) Does the reaction proceed as written?
(b) Under what glucose 1-phosphate concentration could the reaction proceed spontaneously?