Chem 452 - Lecture 4
Enzymes
111010

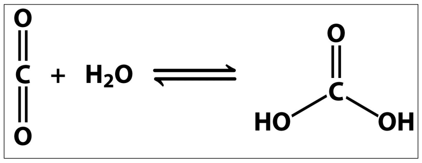
Enzymes are biological catalysts. Nearly every reaction that takes place in a living cell is catalyzed by an enzyme. Most enzymes are proteins. Beside their role in speeding up the rates of chemical reactions, enzymes also play an important role in controlling the flow of material through the myriad of metabolic pathways required to sustain a living cell.

Introduction

- Enzymes are highly effective catalysts.
  - Greatly enhance the rates of reactions
  - Can be very selective
- Important themes
  - Thermodynamics of enzyme catalyzed reactions
  - Transition state stabilization
  - The Michaelis-Menton Model
  - The regulation of enzyme activity

Enzymes are Powerful and Highly Selective

- Example: Hydration of CO_2
  - Carbonic anhydrase increases the rate 8 million-fold.
  - Each Carbonic anhydrase molecule catalyzes 1 million reactions per second!



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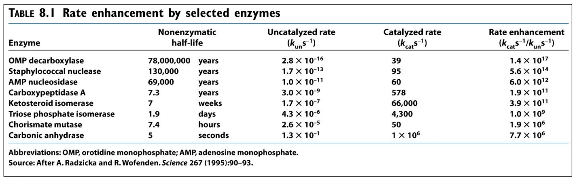

TABLE 8.1 Rate enhancement by selected enzymes

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: After A. Radzicka and R. Wofenden. *Science* 267 (1995):90–93.

Powerful and Highly Selective Catalysts

- Enzymes can be very selective
  - In the types of reactions they catalyze
  - In the reactants (substrates) they accept
  - In the products they form

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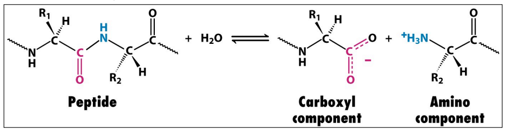

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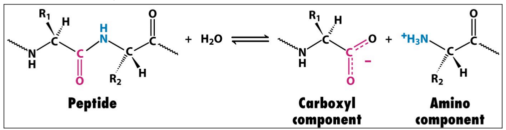
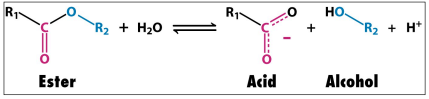
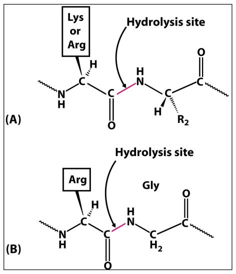




Powerful and Highly Selective Catalysts

- Enzymes can be very selective
  - Substrate specificity
    - Papain
      - L-amino acids
    - Trypsin
      - L-Arg & L-Lys
    - Thrombin
      - L-Arg-Gly



Powerful and Highly Selective Catalysts

- Enzymes sometimes need help
  - Cofactors
    - Metal ions
    - Coenzymes (Organic molecules)

apoenzyme + cofactor = holoenzyme

Powerful and Highly Selective Catalysts

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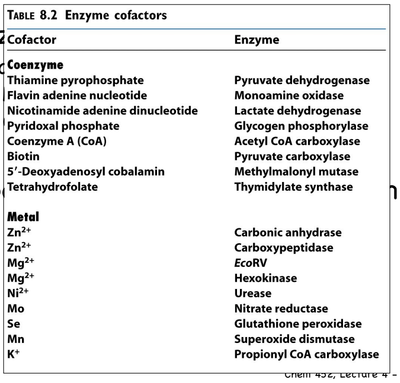

Table 8.2 Enzyme cofactors

Cofactor	Enzyme
Coenzyme	
Thiamine pyrophosphate	Pyruvate dehydrogenase
Flavin adenine nucleotide	Monoamine oxidase
Nicotinamide adenine dinucleotide	Lactate dehydrogenase
Pyridoxal phosphate	Glycogen phosphorylase
Coenzyme A (CoA)	Acetyl CoA carboxylase
Biotin	Pyruvate carboxylase
5′-Deoxyadenosyl cobalamin	Methylmalonyl mutase
Tetrahydrofolate	Thymidylate synthase
Metal	
Zn^{2+}	Carbonic anhydrase
Zn^{2+}	Carboxypeptidase
Mg^{2+}	*Eco*RV
Mg^{2+}	Hexokinase
Ni^{2+}	Urease
Mo	Nitrate reductase
Se	Glutathione peroxidase
Mn	Superoxide dismutase
K^{+}	Propionyl CoA carboxylase

Thermodynamics

The cardinal rules for a chemical reaction, $aA + bB = cD + dD$:

- A reaction occurs spontaneously only if $\Delta G < 0$ (**exergonic reactions**)
  $aA + bB \rightarrow cD + dD$

Thermodynamics

The cardinal rules for a chemical reaction, $aA + bB = cD + dD$:

- A reaction is at equilibrium if $\Delta G = 0$
  $aA + bB \leftrightarrow cC + dD$

Thermodynamics

The cardinal rules for a chemical reaction, aA + bB = cD +dD:

- A reaction is non-spontaneous if ΔG > 0 (**endergonic reactions**)
  aA + bB ← cC+ dD
- An **input** in free energy is required to make non-spontaneous reactions spontaneous.

Thermodynamics

The cardinal rules for a chemical reaction, aA + bB = cD +dD:

- ΔG depends only on the initial (A + B) and final states (C + D) of a reaction, not on the pathway used to get from A + B to C + D.

Thermodynamics

The cardinal rules for a chemical reaction, aA + bB = cD +dD:

- ΔG provides no information about the the rate of a reaction, it only indicates which way a reaction will proceed spontaneously.

Thermodynamics

- The free energy change for a reaction, ΔG, is related to the concentrations of the products and the reactants:

$$\Delta G = \Delta G^{o} + RT\ln\left(\frac{[\mathrm{C}]^{c}[\mathrm{D}]^{d}}{[\mathrm{A}]^{a}[\mathrm{B}]^{b}}\right)$$

where ΔG^{o} is the standard free energy change

Thermodynamics

- The standard free energy change for a reaction, ΔG°, is the free energy change for a reaction carried out under standard conditions
  - For solutions, [A] = [B] = [C] = [D] = 1 M
  - For the solvent, $[H_2O] = 1$
  - For gases, pA = 1 atm
  - T = 25°C

Thermodynamics

- Biochemists modify the definition of the standard state:
  - For solutions, $[H^+] = 10^{-7}$ M (pH 7) instead of $[H^+] = 1$M.
  - This is indicated by $\Delta G^{\circ\prime}$ and $\Delta G'$.

Thermodynamics

- $\Delta G'^\circ$ is related to the equilibrium constant for a reaction.

$$\Delta G' = 0 \text{ (at equlibrium)}$$

$$0 = \Delta G^\circ{}' + RT\ln\left(\frac{[\mathrm{C}]^c[\mathrm{D}]^d}{[\mathrm{A}]^a[\mathrm{B}]^b}\right)_{eq}$$

$$\Delta G^\circ{}' = -RT\ln\left(\frac{[\mathrm{C}]^c[\mathrm{D}]^d}{[\mathrm{A}]^a[\mathrm{B}]^b}\right)_{eq}$$

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$$\Delta G^\circ{}' = -RT\ln\left(\frac{[\mathrm{C}]^c[\mathrm{D}]^d}{[\mathrm{A}]^a[\mathrm{B}]^b}\right)_{eq}$$

$$= -RT\ln\left(K'_{eq}\right)$$

Thermodynamics

- $\Delta G'^\circ$ is related to the equilibrium constant for a reaction.

$$\Delta G^{\circ\,\prime} = -RT\ln\left(K'_{eq}\right)$$
$$= -2.303RT\log\left(K'_{eq}\right)$$
$$K'_{eq} = 10^{\left(-\Delta G^{\circ\prime}/2.303RT\right)}$$
$$= 10^{\left(-\Delta G^{\circ\prime}/5.69\ \text{kJ/mol}\right)}\ \text{at}\ T = 298\ \text{K}$$

Thermodynamics

- $\Delta G'^\circ$ um

const

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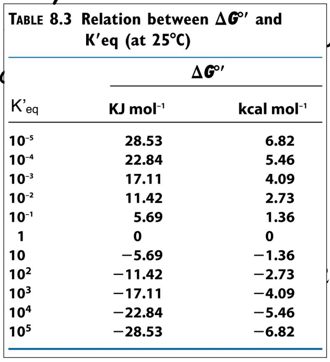


Table 8.3 Relation between $\Delta G^{\circ\prime}$ and $K'eq$ (at 25°C)

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

Problem

Calculate the standard free energy change, $\Delta G^{\circ\prime}$, for the following reaction from its equilibrium constant, K'_{eq}:

aspartate
aminotransferase

glutamate + oxaloacetate $\rightleftharpoons$ aspartate+ α-ketoglutarte

$K'_{eq} = 6.8$

Problem

Calculate the equilibrium constant, K'_{eq}, for the following reaction from its standard free energy change, $\Delta G^{\circ\prime}$:

glucose 6-phosphatase

glucose 6-phosphate + H_2O $\rightleftharpoons$ glucose + P_i

$\Delta G^{\circ\prime} = -13.8$ kJ/mol

Problem

Consider the following reaction:

$$\text{fructose 6-phosphate} \xrightleftharpoons{\text{glucose 6-phosphate isomerase}} \text{glucose 6-phosphate}$$

$$K'_{eq} = 1.97$$

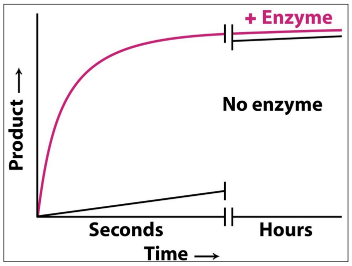
A) What is ΔG°′ for this reaction at 25°C?

B) If the concentration of fructose 6-phosphate is set at 1.5 mM and that for glucose 6-phosphate is set at 0.5 mM, what is ΔG′?

C) Why are ΔG′° and ΔG′ different?

Thermodynamics

- Enzymes (as catalysts) affect only the rate of a reaction, not the ΔG.



Thermodynamics

- Enzymes (as catalysts) affect only the rate of a reaction, not the ΔG.
  - Therefore, enzymes affect the forward and back rates equally:

$$S \leftrightarrow P$$

$$Rate_{\text{forward}} = k_f[S]$$
$$Rate_{\text{back}} = k_b[P]$$

at equilibrium

$$Rate_{\text{forward}} = Rate_{\text{back}}$$
$$k_f[S] = k_b[P]$$
$$\left(\frac{[P]}{[S]}\right)_{eq} = \frac{k_f}{k_b}$$
$$K_{eq} = \frac{k_f}{k_b}$$

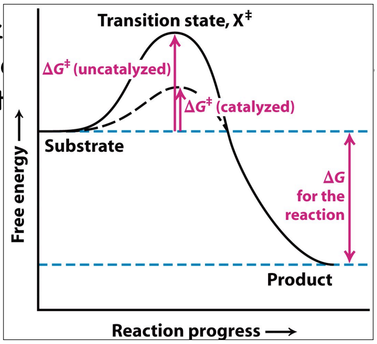
Thermodynamics

- Enzymes accelerate the rates of reactions by stabilizing the transition state

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

Thermodynamics

- Enz rea stat ition



Thermodynamics

- Enzymes accelerate the rates of reactions by stabilizing the transition state

$$S \overset{K^\ddagger}{\rightleftarrows} X^\ddagger \xrightarrow{k} P$$

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

$$V \propto \left[X^\ddagger\right]$$

$$V = k\left[X^\ddagger\right] = \frac{kT}{h}[S]e^{-\Delta G^\ddagger/RT}$$

  - Enzyme speed up reactions by lowering ΔG‡, the activation free energy.

Thermodynamics

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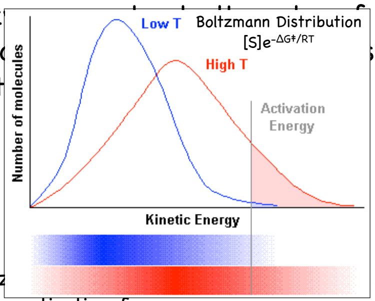

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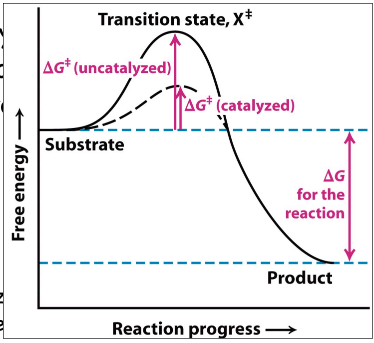

  - Enz $\Delta G‡$,
    the

Thermodynamics

"I think that enzymes are molecules that are complementary in structure to the activated complexes of the reactions that they catalyze, that is, to the molecular configuration that is intermediate between the reacting substance and the products of reaction for these catalyzed processes. The attraction of the enzyme molecule for the activated complex would thus lead to a decrease in its energy and hence to the decrease in the energy of activation of the reaction and to the increase in the rate of the reaction."

- Linus Pauling (Nature 161 (1948):707-709)

Next up

- Enzymes (Chapter 8)
  - Michaelis-Menten model for enzyme catalzyed reactions.