Chem 452 – Lecture 4
Enzymes
Part 1

Question of the Day: Enzymes are biological catalysts. Based on your general understanding of catalysts, what does this statement imply about enzymes?
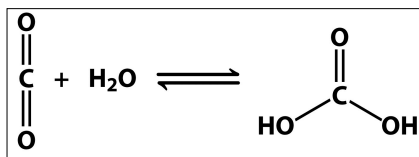

Introduction

Enzymes are very good at what they do:

+ As highly effective catalysts;
  + They greatly enhance the rates of reactions.
  + They can be incredibly selective.

+ Important themes we will examine;
  + Thermodynamics of enzyme catalyzed reactions
  + Catalysis by transition state stabilization
  + The Michaelis–Menten Model for an enzyme catalyzed reaction
  + 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!

$$O=C=O + H_2O \rightleftharpoons HO-C(=O)-OH$$

Enzymes are Powerful and Highly Selective

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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.
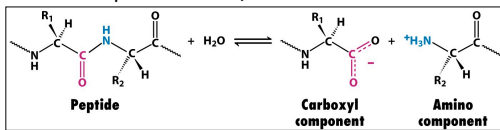
Powerful and Highly Selective Catalysts

* Enzymes can be incredibly selective;
  * In the types of reactions they catalyze
  * In the reactants (substrates) they accept
  * In the products they form
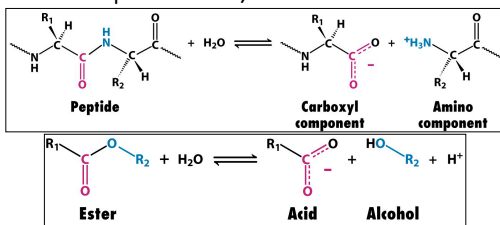

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Peptide   + H_2O ⇌   Carboxyl component   + ^+H_3N Amino component
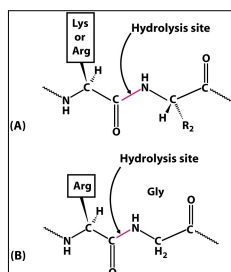

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Ester R_2 + H_2O ⇌   Acid   +   HO R_2 + H^+ Alcohol

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

(A) Lys or Arg — Hydrolysis site

(B) Arg — Hydrolysis site — Gly

Powerful and Highly Selective Catalysts

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

apoenzyme + cofactor = holoenzyme

Powerful and Highly Selective Catalysts

+ Enz
  + Co
    +
    +

apo

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

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Tetrahydrofolate	Thymidylate synthase
Metal	
Zn^{2+}	
Zn^{2+}	
Mg^{2+}	
Mg^{2+}	Hexokinase
Ni^{3+}	Urease
Mo	Nitrate reductase
Se	Glutathione peroxidase
Mn	Superoxide dismutase
K^+	Propionyl CoA carboxylase

Do any of the names of the names of these coenzymes look familiar to you?

Powerful and Highly Selective Catalysts

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Powerful and Highly Selective Catalysts
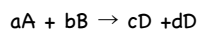
+ **Enzymes sometimes need help**
  + Cofactors
    + Metal ions
    + Coenzymes (Organic molecules)

apoenzyme + cofactor = holoenzyme

Thermodynamics

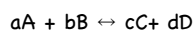The cardinal rules for a chemical reaction, aA + bB = cD +dD:

+ 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:

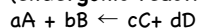+ A reaction is at equilibrium if $\Delta G = 0$

aA + bB $\leftrightarrow$ cC+ dD

Thermodynamics

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

+ A reaction is non-spontaneous if $\Delta G > 0$

  (**endergonic reactions**)
  aA + bB $\leftarrow$ 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:

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

where ΔG^o is the standard free energy change

Thermodynamics

+ The standard free energy change for a reaction, $\Delta G°$, 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 $\Delta G^\prime$.

Thermodynamics

+ $\Delta G^{\prime\circ}$ is related to the equilibrium constant for a reaction.

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

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

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

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$$= -RT \ln\left(K^\prime_{eq}\right)$$

Thermodynamics

+ $\Delta G^{\prime\circ}$ is related to the equilibrium constant for a reaction.

$$\Delta G^{\circ\prime} = -RT \ln\left(K^\prime_{eq}\right)$$

$$= -2.303 RT \log\left(K^\prime_{eq}\right)$$

$$K^\prime_{eq} = 10^{(-\Delta G^{\circ\prime}/2.303RT)}$$

$$= 10^{(-\Delta G^{\circ\prime}/5.71 \text{ kJ/mol})}$$

$$\text{at } T = 298 \text{ K}$$

Thermodynamics

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

K'_{eq}	$\Delta G^{\circ\prime}$	
	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
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Excess reactants at equilibrium

Thermodynamics

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Excess reactants at equilibrium

Excess products at equilibrium

Thermodynamics

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

$$\Delta G^{\circ\prime} = -RT \ln\left(K'_{eq}\right)$$

$$= -2.303 RT \log\left(K'_{eq}\right)$$

$$K'_{eq} = 10^{(-\Delta G^{\circ\prime}/2.303RT)}$$

$$= 10^{(-\Delta G^{\circ\prime}/5.71\ \text{kJ/mol})}$$

at $T = 298$ K

Problem

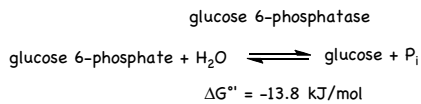Calculate the standard free energy change, $\Delta G°'$, for the following reaction from its equilibrium constant, K'_{eq}:
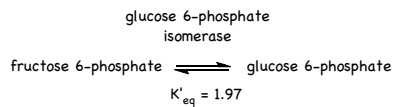
$$\text{glutamate + oxaloacetate} \underset{}{\overset{\text{aspartate} \atop \text{aminotransferase}}{\rightleftharpoons}} \text{aspartate} + \alpha\text{-ketoglutarte}$$
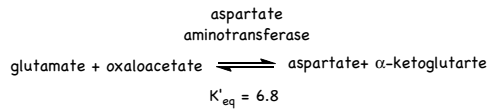
$$K'_{eq} = 6.8$$

Problem

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

$$\text{glucose 6-phosphate + H}_2\text{O} \underset{}{\overset{\text{glucose 6-phosphatase}}{\rightleftharpoons}} \text{glucose + P}_i$$

$$\Delta G°' = -13.8 \text{ kJ/mol}$$

Problem

Consider the following reaction:

$$\text{fructose 6-phosphate} \underset{}{\overset{\text{glucose 6-phosphate} \atop \text{isomerase}}{\rightleftharpoons}} \text{glucose 6-phosphate}$$
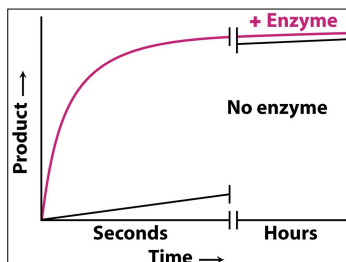
$$K'_{eq} = 1.97$$

A) What is $\Delta G°'$ 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 $\Delta G'$?

Thermodynamics

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

Thermodynamics and Enzymes

+ Enzymes, as catalysts, **have no effect** on the thermodynamics of a reaction.

Thermodynamics

+ Enzymes, as catalysts, affect only the rate of a reaction, **not the ΔG**.

  + Consequently, an affects they have on the kinetics of a reaction must must be equal in both the forward and back reactions:

$$S \leftrightarrow P$$

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

at equilibrium

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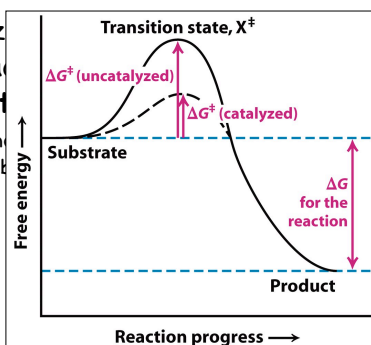$$K_{eq} = \frac{k_f}{k_b}$$

Enzymes and Kinetics

+ Enzymes accelerate the rates of reactions by stabilizing the **transition state** in a reaction

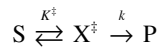 + The **transition state** lies between the substrate and products of a reaction.

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

Enzymes and Kinetics

+ Enz reac **sition** stat

  + Th sub

Thermodynamics

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

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

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

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

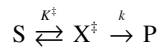$$V = k\left[X^{\ddagger} \right] = \frac{kT}{h}[S]e^{-\Delta G^{\ddagger}/RT}$$

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

Thermodynamics

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

$$\Delta G^{\ddagger} = G_{X^{\ddagger}} - G_{S} \quad \text{activation free energy}$$

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

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Thermodynamics

✦ En f
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  ✦ E g ΔG‡,
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Thermodynamics

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  ✦ Enzyme speed up reactions by lowering ΔG‡,
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Thermodynamics

* Enzy... reac... tion state...

Transition state, X‡

$\Delta G^{\ddagger}$ (uncatalyzed)

$\Delta G^{\ddagger}$ (catalyzed)

Substrate

Free energy →

ΔG for the reaction

Product

Reaction progress →

* Enz... $\Delta G^{\ddagger}$, the activation free energy.

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.