BIO-212 – Kinetics and Catalysis – Exercise session 13
11.12.2025

1) True/False

Which of the following statements about enzyme-substrate binding is correct?

A) Enzyme-substrate binding is always an irreversible process.
B) Enzymes bind substrates through a covalent bond in most reactions.
C) The enzyme's active site is specific for a one or multiple substrates.
D) Substrate binding occurs at random sites on the enzyme.

What is the primary function of an enzyme in a chemical reaction?

A) To stabilize the transition state, leading to a higher activation energy of the reaction.
B) To decrease the activation energy, thereby increasing the reaction rate.
C) To change the equilibrium constant of the reaction to favour substrate formation.
D) To bind its substrate with high affinity, forming a stable enzyme-substrate complex.

In Michaelis-Menten kinetics, what happens to the reaction rate when the substrate concentration is much higher than the Michaelis constant ([S]>>K_m)?

A) The reaction rate increases linearly with increasing substrate concentration.
B) The reaction rate is directly proportional to the substrate concentration.
C) The reaction rate is at its maximum value
D) The reaction rate is slower than at higher substrate concentrations due to substrate inhibition.
Note: Substrate inhibition is a common regulatory mechanism of enzymes, however it is a deviation of classical Michaelis-Menten kinetics.

Which of the following statements about enzyme inhibitors is true?

A) Competitive inhibitors decrease the V_{max} of the enzyme.
B) Non-competitive inhibitors increase the K_m, but do not affect the V_{max}.
C) Competitive inhibitors can be partially overcome by increasing substrate concentration.
D) Non-competitive inhibitors bind to the enzyme-substrate complex, leading to an increase of the K_m and a decrease of the V_{max}.

2) Effects of enzyme catalysis

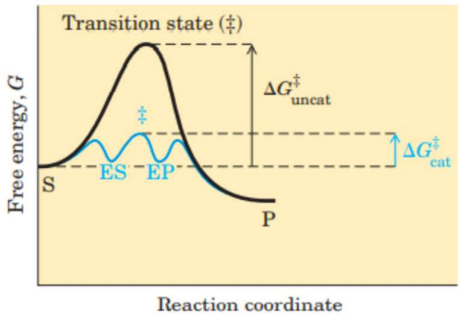
Which of the following effects would be brought about by an enzyme catalysing the simple reaction:

$$S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} P \qquad \text{Where} \qquad K'_{eq} = \frac{[P]}{[S]}$$

a) Decreased K'_{eq}
b) Increased k_1
c) Increased $\Delta G^{\ddagger}$ (E_A)
d) Decreased $\Delta G^{\ddagger}$ (E_A)
e) Increased K'_{eq}
f) Decreased $\Delta G'^{o}$
g) Increased k_2

Answer:

b), d) and g)



3) First-order kinetics

You are studying the kinetics of different compounds containing radioactive elements, with particular interest in iodine-123 (^{123}I) which is important for medical imaging of thyroid function and follows a **first-order decay kinetics** into ^{123}Te. A 15 µg sample of ^{123}I has decreased to 7.5 µg after 13 hours. After how much time will it decay to less than 0.95 µg?

$$^{123}I \rightarrow {}^{123}Te$$

Answer:

Time of half-life ($t_{1/2}$) = 13 hours so decrease by half every 13 hours as this is first order kinetics. You can then use the equation describing first order kinetics:

$[^{123}I_t] = [^{123}I_0] * e^{-k\,t} = [^{123}I_0] * e^{-0.693\,t\,/\,t1/2}$

If you solve for t:

$t = \ln([^{123}I_t] / [^{123}I_0]) / (-0.693/t_{1/2}) = -2.759 / -0.053 =$ **52.05 hours**

4) Degradation of H_2O_2

a. During aerobic metabolism reactive oxygen species (ROS) may be formed, among them hydrogen peroxide (H_2O_2). The enzyme ***Catalase*** is found in most aerobic organisms where it catalyzes the following reaction:

$$2\,H_2O_2 \rightarrow 2\,H_2O + O_2$$

Can you reason why it is prerogative for organisms to prevent the accumulation of hydrogen peroxide?

Hydrogen peroxide is a strong oxidizer. It produces hydroxyl radicals which may react with cellular components such as DNA, lipids or proteins. As such it interferes with most cellular processes by impairing enzyme function and DNA and membrane integrity.

b. The uncatalyzed decomposition of hydrogen peroxide as shown above proceeds spontaneously, as it has a negative Gibbs free energy under standard conditions in aqueous solution.

How does the presence of Catalase impact the Gibbs free energy of the reaction? Can you reason why aerobic organisms rely on the enzymatically catalyzed degradation of hydrogen peroxide over just spontaneous decomposition?

A catalyst does not impact the Gibbs free energy of a reaction. Instead, it lowers the energy of the high-energy transition state(s) through which the reaction proceeds. For this reason, the rate constant of a reaction is increased by the presence of a catalyst. The spontaneous decomposition of H_2O_2 is too slow for aerobic organisms and would result in the accumulation of reactive oxygen species, causing oxidative damage.

c. In a laboratory course, you are tasked with measuring the activation energy of the degradation of hydrogen peroxide when catalyzed by catalase. For this purpose, you add purified catalase to a solution containing hydrogen peroxide. You follow the reaction by measuring the rate of oxygen formation immediately after mixing. The following rate constants were determined at two temperatures:

Temperature (K)	Rate constant k (s^{-1})
293	7
303	8

i. **Transform the Arrhenius equation into linear form and complete the following table using the values provided above.**
(Hint: apply a ln() transformation to both sides of the equation.)

1/T	ln(k)
1/293	ln(7)
1/303	ln(8)

$$k = A * e^{-\frac{E_A}{RT}}$$

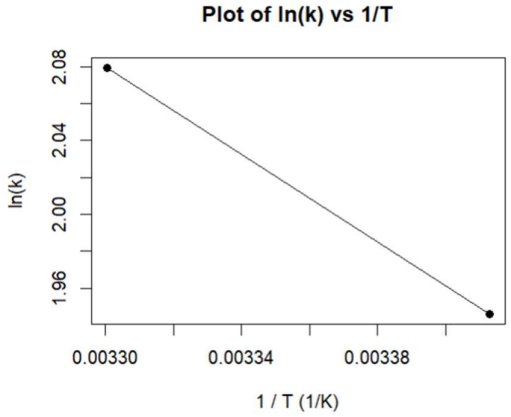
$$\ln(k) = \ln(A * e^{-\frac{E_A}{RT}})$$

$$\ln(k) = \ln(A) + \ln(e^{-\frac{E_A}{RT}})$$

$$\ln(k) = \frac{-E_A}{R} * \frac{1}{T} + \ln(A)$$

ii. You want to calculate the activation energy (E_a) using the linearized Arrhenius equation. **You may assume the activation energy (E_a) and the pre-exponential factor (A) to be constants.**
Plot ln(k) against 1/T. Why does this result in a straight line?

Answer:



Since the activation energy and the pre-exponential factor are constants, the resulting plot must be a straight line. The y-intercept is provided by ln(A), the slope of the line by $\frac{-E_A}{R}$. Therefore, the activation energy may be derived from the slope of the equation.

iii. Using your plot, derive the activation energy (E_a).

The slope of a linear equation is defined as:

$$m = \frac{\Delta y}{\Delta x}$$

Adapted to our example:

$$m = \frac{\ln(k_2) - ln(k_1)}{\frac{1}{T_2} - \frac{1}{T_1}} = \frac{\ln(8) - \ln(7)}{\frac{1}{303K} - \frac{1}{293K}} = -1185K$$

The slope is defined as

$$m = \frac{-E_A}{R}$$

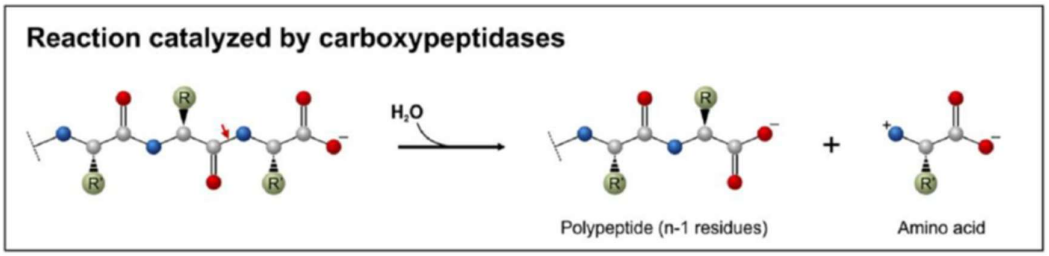
By solving for E_A we get

$$E_A = -m * R = 1185K * 8.314 \frac{J}{mol * K} = 9.85\ kJ/mol$$

Important caveat:

Catalase is a diffusion-limited enzyme. This means the speed of the reaction is determined by how fast substrates and products diffuse in and out of the catalytic center. This makes the pre-exponential factor A temperature-dependent, so assuming it is constant is a simplification.

5) Carboxypeptidase



The enzyme carboxypeptidase cleaves carboxy-terminal amino acids from its peptide substrates. Carboxypeptidase is constituted by a single peptide chain of 307 amino acids. The two catalytic residues of carboxypeptidase are Arg-145 and Glu-270.

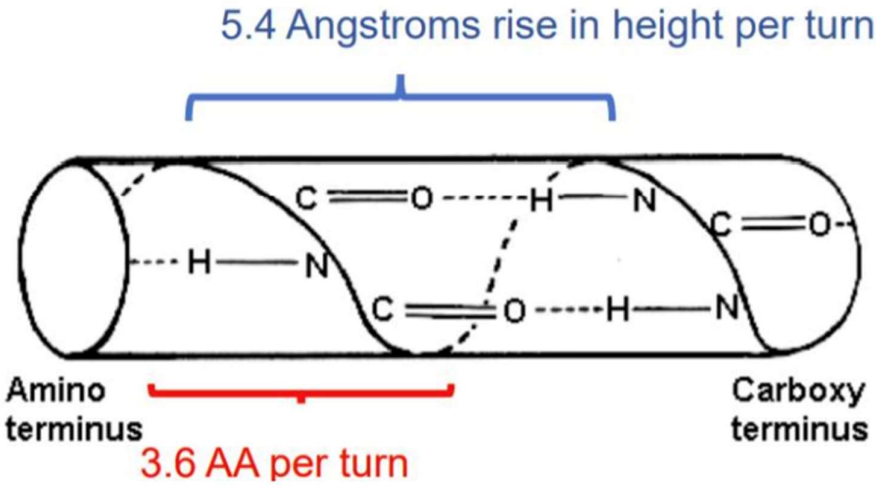
a) If carboxypeptidase was a perfect alpha helix what would the distance between these amino acids be in Å?

b) Explain how these two amino acids, distant from each other in the primary structure, can nevertheless catalyse a reaction taking place in the range of few nanometers.

c) If only these 2 amino acids are involved in catalysis, why is carboxypeptidase constituted by 307 residues?

Answers:

a)



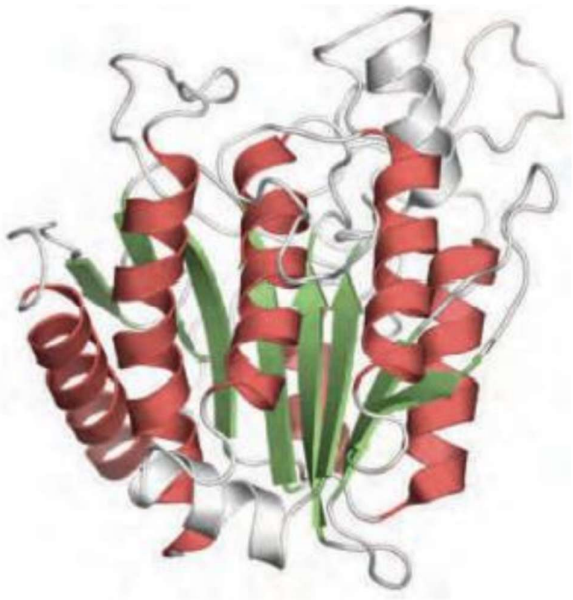
5.4Å per helical turn

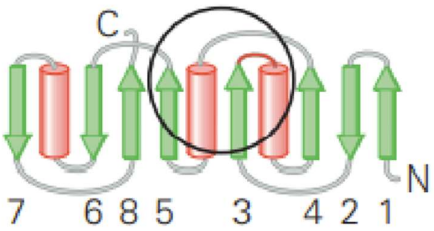
3.6 amino acids per helical turn

5.4/3.6 = 1.5Å per amino acid
270-145 = 125 → There are 125 amino acids between Arg-145 and Glu-270
125 x 1.5Å = 187.5Å → Distance between the 2 residues if the protein was a perfect α-helix

b) A polypeptide chain folds into a complex 3-dimensional structure. Therefore, despite being distant in primary structure, two amino acids may be in close vicinity in the tertiary structure of the protein.

c) The rest of the protein sequence is important to maintain the folding, bringing the catalytic amino acids closer together and orienting them accordingly. Furthermore, the catalytic residues are embedded in the substrate-binding pocket, which contains additional amino acids.





6) Michaelis Menten Kinetics

a) At what substrate concentration would an enzyme with a k_{cat} of 30.0 s^{-1} and a K_m of 0.008 mol/L operate at one-fifth of its maximum rate?

b) Determine the fraction of the maximum reaction rate (V_o/V_{max}) that would be obtained at the following concentrations of [S]: 0.5 K_m, 2 K_m, 5 K_m

c) Suppose an **uncompetitive inhibitor** is introduced with a α'=5. How does the inhibitor affect the maximum velocity V_{max} and what is the new V_{max} in the presence of the inhibitor? Assume that the enzyme concentration is 50 nmol/L.

Answers:

a) To find the substrate concentration we use the Michaelis-Menten equation:

$$\frac{V_{max}}{5} = \frac{V_{max}[S]}{K_m + [S]}$$

Solving the equation for [S] → [S] = K_m / 4 = 0.008 / 4 = 0.002 M

b) For [S] = K_m/2:

$$\frac{V_0}{V_{max}} = \frac{\frac{K_m}{2}}{K_m + \frac{K_m}{2}} = \frac{1}{3} = 0.33$$

For [S] = 2 K_m: Fraction of V_{max} = 0.67

For [S] = 5 K_m: Fraction of V_{max} = 0.83

c) V_{max} (without inhibitor) = k_{cat} x $[E_t]$ = 30 x 50 x 10^{-9} = 1.5 x 10^{-6} M s^{-1}

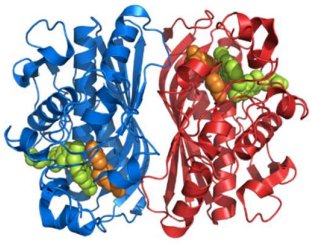
With noncompetitive inhibition, when the Km << [S] we can approximate the formula:

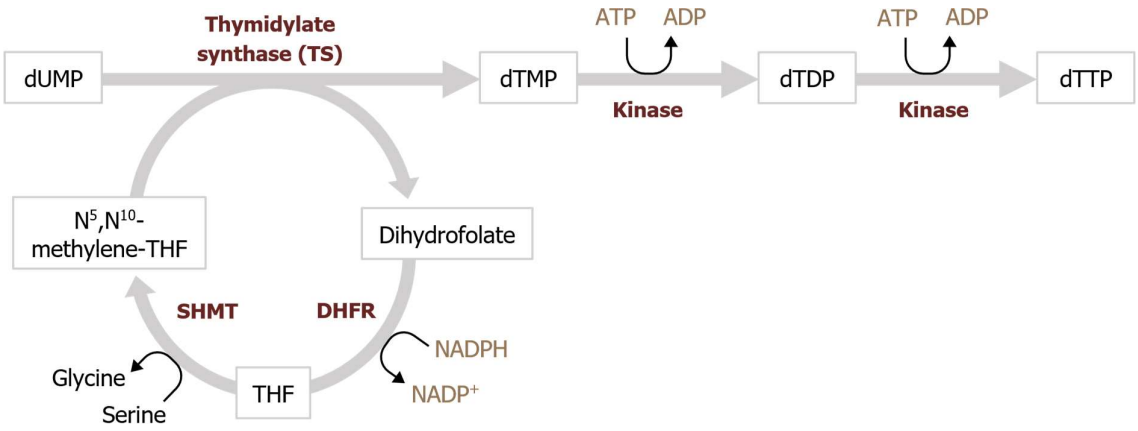
$$V_0 = \frac{V_{max}[S]}{K_m + \alpha'[S]}$$

To Vmax apparent = Vmax/ α’

$V'_{max} = 1.5/5 = 3 \times 10^{-7}$ M s^{-1}

7) Thymidylate Synthase Kinetics





The enzyme thymidylate synthase (TS) catalyzes the reductive methylation of 2'-deoxyuridine 5'-monophosphate (dUMP) to 2'-deoxythymidine 5'-monophosphate (dTMP), a key building block for DNA. This reaction is crucial for DNA replication and repair, making TS an important target in cancer treatment. Researchers have found that the K_m for dUMP is 4μM and the kcat of the enzyme is 20 min^{-1}.

a) In an experiment, the [dUMP] used was 6mM, and the initial velocity (V_0) was 480 nM min^{-1}. What was the concentration of TS used in in this experiment?

b) In a second experiment, the enzyme concentration [TS] is 0.5 μM and V_0 = 5 μM min^{-1}. What was the [dUMP] in this experiment?

c) You are developing a new chemotherapic drug that acts as a competitive inhibitor of TS with an α = 10. This inhibitor is added to a mixture of enzyme and substrate, resulting in a V_0 of 240 nM min^{-1}. At an enzyme concentration of 24 nM, which dUMP concentration has been used in the experiment?

Answers:

a)

$$V_{max} = k_{cat}[E_t]$$

$$V_0 = \frac{k_{cat}[E_t][S]}{K_m + [S]}$$

$$[E_t] = \frac{V_0(K_m + [S])}{k_{cat}[S]}$$

$E_t = 480 \times (4 \times 10^{-6} + 6 \times 10^{-3}) / (20 \times 6 \times 10^{-3}) = 24nM$

b)

$$V_0 = \frac{k_{cat}[E_t][S]}{K_m + [S]}$$

rearrange the equation to solve for [S]

$[S] = 5 \times 10^{-6} \times 4 \times 10^{-6} / (20 \times 0.5 \times 10^{-6} - 5 \times 10^{-6}) = 4\mu M$

c)

$$V_0 = \frac{k_{cat}[E_t][S]}{K_m \alpha + [S]}$$

$[S] = 240 \times 10^{-6} \times 4 \times 10^{-6} \times 10 / (20 \times 24 \times 10^{-9} - 240 \times 10^{-6}) = 40\mu M$