**Anglo-Chinese Junior College**
**Class of H3 Chemistry '26**

# Enzyme Catalysis tutorial

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Special thanks to H3 chemistry teachers


Binding Mechanisms

1. ATP synthase is an important enzyme in the functioning of a large portion of living things. It rotates between 3 different states, of which one of them catalyses the spontaneous formation of ATP at a very high rate, following this equation:

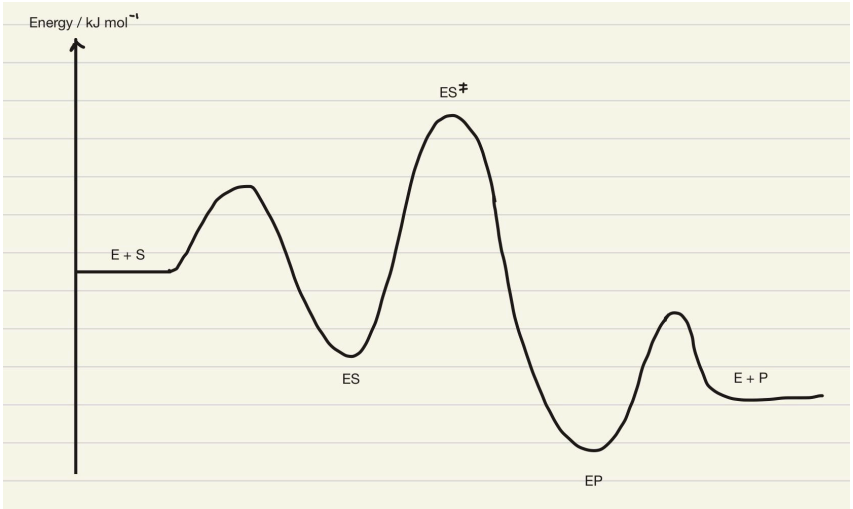
$$ADP + P_i \rightarrow ATP$$

Given that ADP and $P_i$ are in excess, what binding mechanism is ATP synthase likely to use, and why?

(A) Uniform binding, because ADP and $P_i$ concentrations are high.
(B) Uniform binding, because ADP and $P_i$ concentrations are low.
(C) Differential binding, because ADP and $P_i$ concentrations are high.
(D) Differential binding, because ADP and $P_i$ concentrations are low.

[1]

2. Given the following energy profile diagram representing the reaction pathway of an enzyme via the uniform binding mechanism, draw another line representing the reaction pathway of the same enzyme undergoing differential binding. You may use the symbols E, S and P to represent the enzyme, substrate and products respectively.

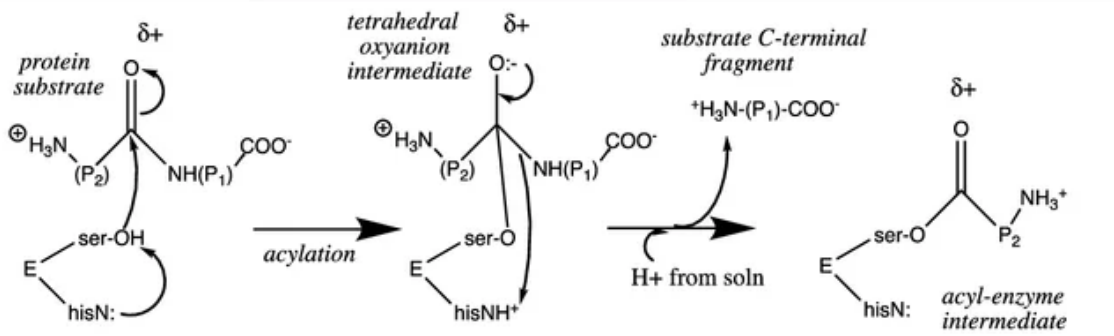



[4]

Types of Enzyme Interactions

3. The enzyme chymotrypsin catalyses the lysis of polypeptides into amino acids, and its mechanism is described below.

From the diagram, identify and explain the mechanisms of catalysis that can be observed.



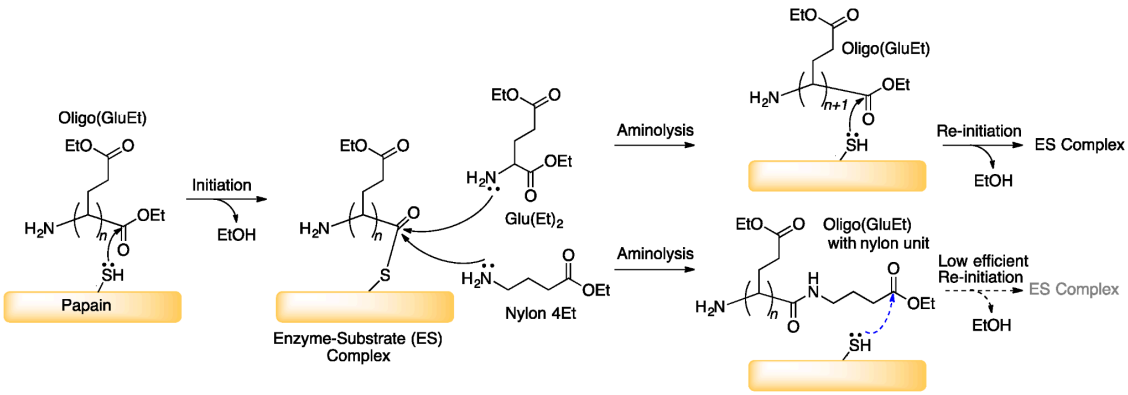

[4]

4.

Transition state theory

4. The enzyme papain catalyses the polymerisation of glutamate esters. Papain contains an active-site cysteine residue (–SH) which participates directly in the catalytic cycle.

Answer the following questions.




Two molecules are studied:

- **Molecule A** is a peptide substrate.
- **Molecule B** is a transition state analogue that resembles the tetrahedral transition state formed during catalysis.

The activation free energy for hydrolysis of Molecule A is **68.0 kJ mol$^{-1}$** in the absence of papain and **54.0 kJ mol$^{-1}$** in the presence of papain. Assume the reaction occurs at **298 K**.

(a) Calculate the factor by which papain increases the reaction rate
(b) State the catalytic action that papain undergoes. Hence, draw a labelled reaction energy profile for the papain catalysed hydrolysis of a peptide bond

Michaelis-Menten Kinetics

5. Enzyme A has a $K_m$ of 0.1 mM for its substrate, while Enzyme B has a $K_m$ of 10 mM for the same substrate. Which enzyme has a higher affinity for the substrate?

6. Given that the substrate concentration needed for Amylase to react at half the maximum rate when reacting with alpha-maltosyl fluoride is $4.5mM$, find $Km$.

7. It is known that Catalyse is a biological catalyst which speeds up the decomposition of $H_2O_2$ into $H_2O$ and $O_2$. For a certain concentration of $H_2O_2$, the $V_{max}$ is $2000$ mMmin$^{-1}$ and $K_m$ = $80mM$. Given that $[S] = 100mM$, find $V_0$ [$1110 mM min^{-1}$]

8a. In humans, the reaction between alcohol dehydrogenase (isozyme ADH4) and 1-hydroxymethylpyrene has a $K_m$ value of $0.0283\ mM$. Given that the maximum rate ($V_{max}$) of reaction possible for this enzyme is $51.8\ nmol(min \cdot mg)^{-1}$. When 1mg of enzyme and $0.05mM$ of the substrate reacts, find the initial rate using the Michaelis-Menten equation. [$33.1\ nmol min^{-1}$]

8b. However, this reaction is inhibited competitively with ethanol at a $K_i$ of $3.3\ mM$. Given that the conditions at (a) is constant, find the new initial rate of reaction when $1.0mM$ of ethanol is added. [29.8 nmolmin$^{-1}$]

[Adapted from (Kollock et al., 2007)]