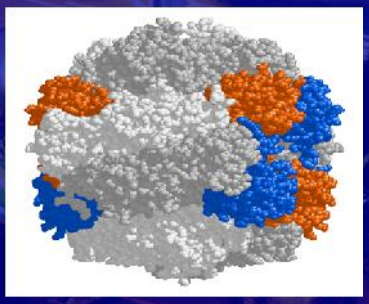
LECTURE 2: INTRODUCTION TO ENZYME

1. **The living cell is the site of tremendous biochemical activity (biochemical reactions) which, in majority, do not take place spontaneously, but require catalysts to occur at normal rates.**



Space-filling view of **RubisCO** showing the arrangement of the **large chain dimers** (white/grey) and the small chains (blue and orange). from the Protein Data Bank

2. **Enzymes are the catalysts of biochemical reactions and responsible for bringing about almost all of the chemical reactions in living organisms.**
3. **The life of organisms would be much different and go on very slow without enzymes**

LECTURE OUTCOMES

After mastering the present lecture materials, students will be able to

1. make an enzyme cleaner for use to remove stains
2. explain enzymes as metabolic catalysts to support certain reactions
3. describe the initial discovery of enzymes and the first crystallization of enzyme
4. explain enzyme properties that include enzyme structure and characteristics

LECTURE FLOW

Competency Project

I. INTRODUCTION
1. Definition
2. Discovery of Enzymes
2.1 Zymase
2.2 Urease
2.3 Papain

II. ENZYME PROPERTIES
A. Enzyme Structure
1. Proteins
2. Cofactors
3. Active Site

B. Enzyme Characteristics
a. Critical cell components
b. Very efficient
c. Reduce ΔG
d. Specific
e. Subject to regulatory control

PROJECT

Enzyme Cleaner

- Try Enzyme cleaner to remove stains

  There are many different types of enzymes used in cleaning solutions, but the primary enzymes proprietary blend most use are Protease, Amylase, and Lipase.

- **Materials**
  1. 300 grams of citrus scraps
  2. 100 grams of brown sugar
  3. 1 liter of water
  4. 2-liter or larger plastic bottle

- **Procedure**
  1. 300 g potongan jeruk ditempatkan dalam wadah (botol)
  2. Tambahkan 100 g gula coklat (brown sugar) ke dalam botol
  3. Tuangkan 1 liter air bersih ke dalam botol kemudian tutup
  4. Kocok campuran bahan hingga gula terlarut dan tercampur dengan rata yang memakan waktu sekitar 30-60 detik
  5. Buat keterangan waktu (hari & bulan) pembuatan bahan pada wadah
  6. Simpan wadah pada tempat aman selama 3 bulan untuk mendapatkan hasil fermentasi yang baik
  7. Bahan kemudian sudah dapat digunakan (300 ml bahan + 1 liter air) dengan kualitas yang sama dengan yang dijual

I. INTRODUCTION

1. Definition

- Enzymes are metabolic catalysts which increase the rate of chemical reactions by lowering the activation energy of that reactions
- An enzymes (in yeast) is made up of **protein** that acts as a **catalyst** to cause certain desired reaction
- Enzymes are the largest and most highly specialized catalysts in the body for the reactions involved in metabolism

2. Discovery of enzymes

2.1 Zymase

- Eduard Buchner
  - Buchner discovered in 1897 that yeast extracts could ferment sugar to alcohol:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + 2\ ATP$$

  - The conversion of sugar to alcohol (ethanol) is catalyzed by several enzymes, called zymase, that are produced by
  - Buchner's finding showed that fermentation was promoted by molecules that continued to function when removed from cells.

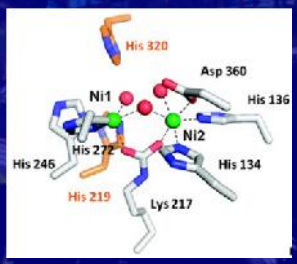
2.2 Urease

- In 1926, Sumner isolated and crystallized jack bean (*Canavalia ensiformis*) urease, which catalyzes the reaction:

$$CH_4N_2O + H_2O \rightarrow 2NH_3 + CO_2$$

Kacang parang (Jack bean, *Canavalia ensiformis*)

Urease active site



Panpae *et al.*, 2012

Crystal structure of the first plant urease from Jack bean (*Canavalia ensiformis*). DOI: 10.2210/pdb3la4/pdb
Classification: HYDROLASE
Deposited: 2010-01-06 **Released**: 2010-07-28
Deposition author(s): Ponnuraj, K.
Organism: *Canavalia ensiformis*

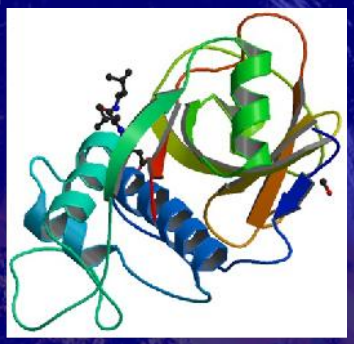
http://www.rcsb.org/pdb/images/3LA4_bio_r_500.jpg?bioNum=1

- The urease crystals contained only protein, leading Sumner to propose that all enzymes are proteins.

2.3 Papain

What is papain?

- Papain is a protein-cleaving enzyme derived from papaya and certain other plants.
- Enzymes are complex molecules produced in living organisms to catalyze (speed up) chemical reactions within the cell



Crystal structure of a papain-E-64 complex

Varughese *et al*. (1989)

Why is meat tough?

- Muscles have to endure a lot of mechanical stress, and are made of strong fibers that are hard to cut, and tough connective tissue holds them together
- Individual muscle cells contain microscopic **fibrils** that give them their structural integrity and allow them to contract.
- The fibrils have a complex internal structure bound together by long protein chains. The connective tissue that holds the muscle together is also mostly protein.

How does papain tenderize meat?

- Papain cuts the protein chains in the fibrils and also in the connective tissue, disrupting the structural integrity of the muscle fiber, and tenderizing the meat.

3. Enzyme Nomenclature

- Most enzymes are named for their substrates and for the reactions that they catalyze, with the suffix "ase" added. (ATPase)
- **Six major classes of enzymes (Superfamilies: EC 1.1.3.4)**

1. Transferases
   - Transfer functional groups between molecules
2. Oxidoreductases
   - Transfer electrons (RedOx reactions)
3. Hydrolases
   - Break bonds by adding H_2O
4. Lyases
   - Elimination reactions to form double bonds
5. Isomerases
   - Intramolecular rearangements
6. Ligases
   - Join molecules with new bonds

- Transferases

  Aminotransferases: transferases catalyzing the amino acid degradation by removing amino groups.

- Oxidoreductases

  Alcohol dehydrogenase: an oxidoreductase converting alcohols to aldehydes/ ketones.

- Hydrolases

  Glucose-6-phosphatase: a hydrolase that removes the phosphate group from glucose-6-phosphate, leaving glucose and H_3PO_4.

- Lyases

  Pyruvate decarboxylase: a lyase that removes CO_2 from pyruvate.

- Isomerases

  Ribulose phosphate epimerase: an isomerase that catalyzes the interconversion of ribulose-5-phosphate and xylulose-5-phosphate.

- Ligases

  Hexokinase: a ligase that catalyzes the interconversion of glucose and ATP with glucose-6-phosphate and ADP.

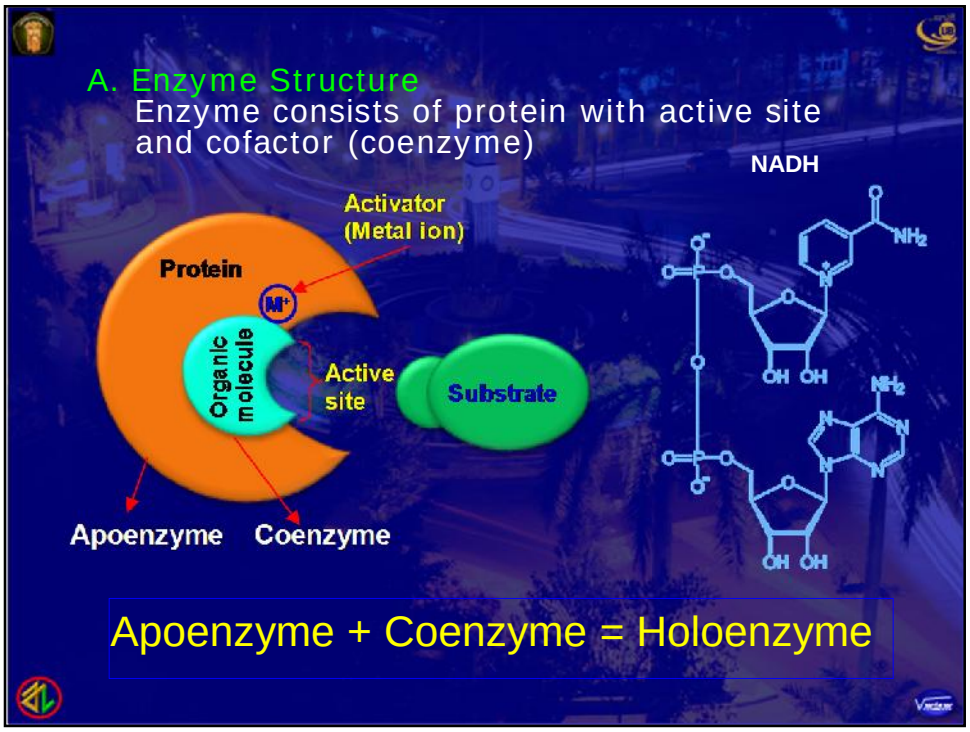
2. ENZYME PROPERTIES

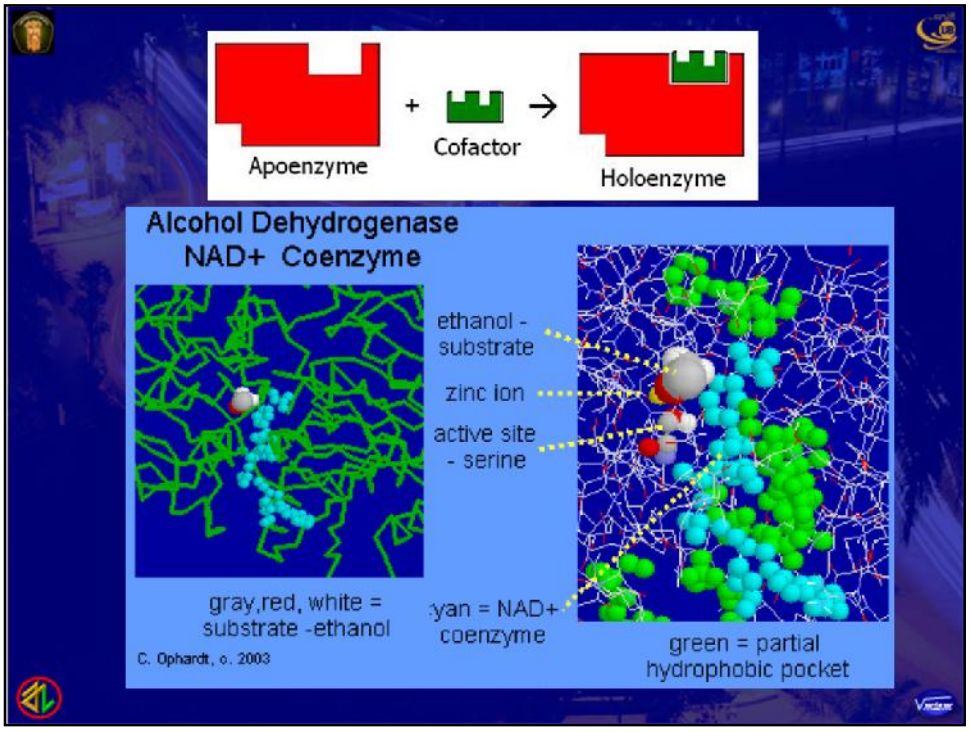
A. Enzyme Structure

1. Proteins
2. Cofactors
3. Active Site

B. Enzyme Characteristics

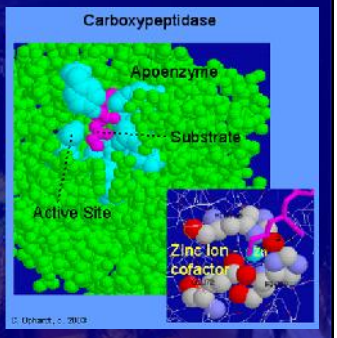
a. Critical components of cells
   Almost all reactions in living organism/plants are catalyzed by enzymes)
b. Very efficient
c. Reduce ΔG for reaction (by binding the transition state)
d. Specific
e. Subject to regulatory control of various sorts


A. Enzyme Structure
Enzyme consists of protein with active site and cofactor (coenzyme)
NADH
Activator (Metal ion)
Protein
Organic molecule
Active site
Substrate
Apoenzyme
Coenzyme
Apoenzyme + Coenzyme = Holoenzyme


Apoenzyme
Cofactor
Holoenzyme
Alcohol Dehydrogenase
NAD+ Coenzyme
ethanol - substrate
zinc ion
active site - serine
gray,red, white = substrate -ethanol
yan = NAD+ coenzyme
green = partial hydrophobic pocket
C. Ophardt, o. 2003

1. Protein

- All known enzymes are high molecular weight compounds that are made up principally of proteins (except for catalytic RNA)
  - Apoenzyme: The polypeptide or protein part of the enzyme which may be inactive in its original synthesized structure
  - The inactive form of the apoenzyme is known as a proenzyme or zymogen.

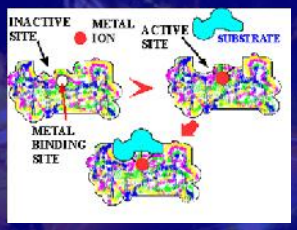


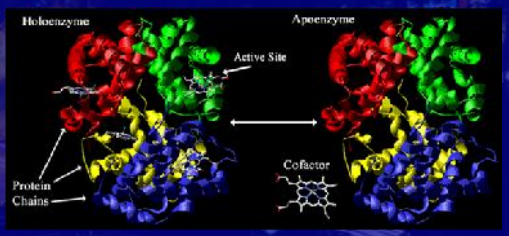
- The proenzyme may contain several extra amino acids in the protein which are removed, and allows the final specific tertiary structure to be formed before it is activated as an apoenzyme.

2. Cofactors

- Many enzymes require the presence of other compounds - cofactors - before their catalytic activity can be exerted
- This entire active complex is referred to as the holoenzyme; apoenzyme (protein portion) + the cofactor (coenzyme, prosthetic group or metal-ion-activator)

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





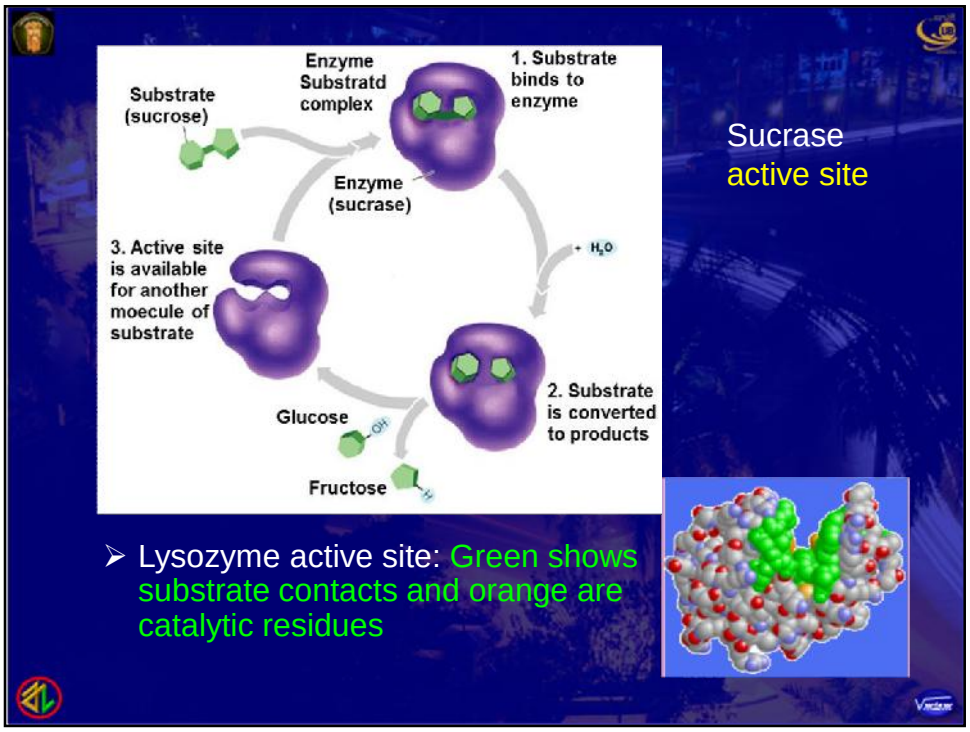
Apoenzyme + Cofactor = Holoenzyme

The cofactor may be:

1. **A coenzyme** - a non-protein organic substance which is loosely bound to the protein part, dialyzable, and thermostable
2. **A prosthetic group** - an organic substance which is firmly bound to the protein or apoenzyme portion, dialyzable and thermostable.
3. **A metal-ion-activator** - these include K^{+}, Fe^{++}, Fe^{+++}, Cu^{++}, Co^{++}, Zn^{++}, Mn^{++}, Mg^{++}, Ca^{++}, and Mo^{+++}

3. Enzyme Active Sites

- The active site is
  - the specific area of the enzyme to which the substrate attaches during the reaction
  - part of the conformation of the enzyme molecule arranged to create a special pocket or cleft whose three-dimensional structure is complementary to the structure of the substrate
- The enzyme and the substrate molecules "recognize" each other through this structural complementarity
- The substrate binds to the enzyme through relatively weak forces -H bonds, ionic bonds (salt bridges), and van der Waals interactions between sterically complementary clusters of atoms.



B. Characteristics

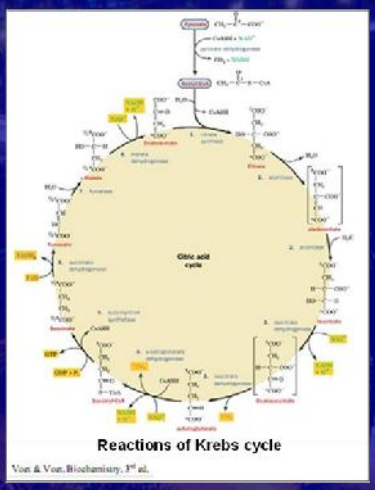
a. Critical Components

Enzymes are critical for every aspect of cellular life (cell metabolism & biological processes)

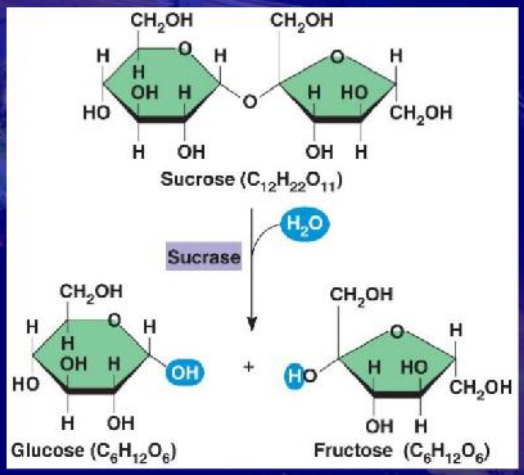
b. Very efficient catalysts

A very small quantity of an enzyme can catalyze the transformation of vastly lager quantity of the substrate

- **Sucrase**
- **Catalase**



- ***Sucrase*** **(*invertase*)** can effect the hydrolysis of at least 1,000,000 times its own weight of sucrose without exhibiting any appreciable diminution in its activity



- ***Catalase*** is one of the more efficient enzymes, one molecule of this enzyme being able to catalyze the conversion 5,000,000 molecules of H_20_2 per minute (the reduction of hydrogen peroxide to water and molecular oxygen) when conditions are favorable

Enzyme	Reaction catalyzed	Function of reaction	Rate *
Catalase	$2H_2O_2 \rightarrow 2H_2O + O_2$	removes toxic H_2O_2 from cell	10^{15}
Carbonic anhydrase	$CO_2 + H_2O \quad H_2CO_3$	hydrates CO_2 gas for transport	10^7

* rate of enzyme catalyzed compared to rate uncatalyzed

c. Activation Energy

- Enzymes reduce the activation energy of a reaction so that the kinetic energy of most molecules exceeds the activation energy required and so they can react.
  - For example, for the catalase reaction

    $2H_2O_2 \rightarrow 2H_2O + O_2$

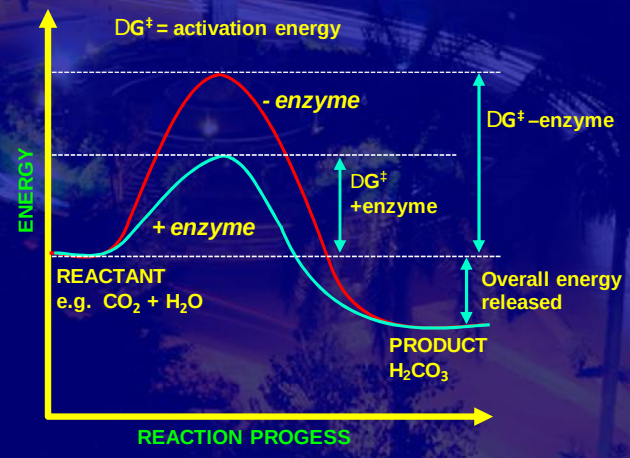
    The activation energy is

    86 kJ mol^{-1} with no catalyst

    62 kJ mol^{-1} with an inorganic catalyst

    1 kJ mol^{-1} with the enzyme catalase

- RATE of reaction is affected by enzyme
- RATE depends on $\Delta G^{\ddagger}$, the Arrhenius activation energy (i.e., the *free energy of activation* for the reaction).



Gibb's Free Energy Change (ΔG)

- Enzymes decrease activation energy ($\Delta G^{\ddagger}$) for reactions catalyzed.
  - K'°eq = [P]/[S]
  - From thermodynamics, the equilibrium constant and the free energy are related by the expression

    $\Delta G'^{\circ} = -RT \ln K'^{\circ}eq$

    $= -2.3RT \log K'^{\circ}eq$
- ΔG = overall difference in free energy between final (P) and starting (S), not affected by enzyme.

K'_{eq}	$\Delta G'^{0}$ (KJ/mol)
10^{-6}	34.2
10^{-5}	28.5
10^{-4}	22.8
10^{-3}	17.1
10^{-2}	11.4
10^{-1}	5.7
1	0
10	-5.7
10^{2}	-11.4
10^{3}	-17.1

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}/k_{un})
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.

Enzymatic catalysts have much higher rates than non-enzymatic catalysts do, and even at relatively low temperatures

Table 1. Examples of the catalytic power of enzymes

Substrate	Catalyst	Temperature (K)	Rate of constant k $(mol\ dm^{-3})^{-1}\ s^{-1}$
Benzamide (hydrolysis)	H^+	325 (52^0C)	2.4×10^{-6}
	OH^-	326 (53^0C)	8.5×10^{-6}
	α-chymotrypsin	298 (25^0C)	14.9
Urea (hydrolysis)	H^+	335 (62^0C)	7.4×10^{-7}
	urease	294 (21^0C)	5.0×10^{6}
			14.9
$2H_2O_2 \rightarrow 2H_2O+O_2$	Fe^{2+}	295 (22^0C)	56
	catalase	295 (22^0C)	3.5×10^{7}

Price & Stevens (1984): Fundamentals of Enzymology

UREA FERTILIZER

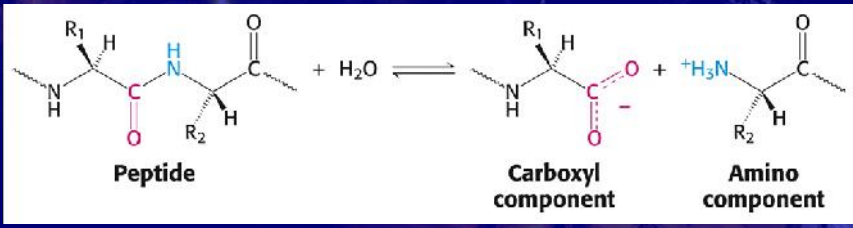
- The optimum conditions for enzyme catalysis are almost invariably moderate temperatures, and pHs which are not extreme
- The contrast between a reaction catalysed by an enzyme and by a non-enzymatic catalyst is well illustrated by the process of nitrogen fixation (i.e. reduction of N2 to ammonia). Nitrogenase catalyses this reaction at temperatures around 300 K and at neutral pH. The enzyme is a complex system comprising two dissociating protein components one of which contains iron and the other iron and molybdenum. Several molecules of ATP are hydrolyzed during the reduction
- By contrast, in the industrial synthesis of ammonia from nitrogen and hydrogen, the conditions used are as follows: temperature 700 - 900 K, pressure 100 - 900 atmospheres, and the presence of an iron catalyst, often promoted by traces of oxides of other metals

d. Enzyme Specificity

Enzymes very specific

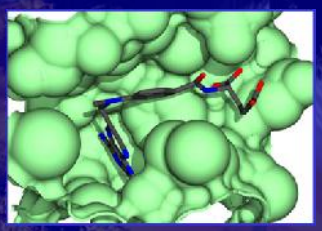
- for substrate acted upon
- for reaction catalyzed

Example: Proteases are a whole class of enzymes that all catalyze hydrolysis of peptide bonds:



Specificity class

- **Absolute specificity** - the enzyme will catalyze only one reaction.
- **Group specificity** - the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.
- **Linkage specificity** - the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.
- **Stereochemical specificity** - the enzyme will act on a particular steric or optical isomer.



Enzyme Specificity

e. Enzyme Regulation

- Enzymes are tightly regulated light switches
- Unregulated enzymes become constitutively active or inactive (light is always on or off)
- Unregulated enzyme activity disrupts cell homeostasis and often lead to disease states.
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