# AN INTRODUCTION TO PROTEINS AND ENZYMES

- **What are Proteins?**
- **Proteins are polymers of amino acids, with each amino acid residue joined to its neighbor by a specific type of covalent bond called peptide bond**
- **Proteins can be very long polypeptide chains of 100 to several thousand amino acid residues.**
- **Some proteins are composed of several noncovalently associated polypeptide chains, called subunits**
- **Simple proteins yield only amino acids on hydrolysis;**
- **Conjugated proteins contain in addition some other component**
- **Twenty different amino acids are commonly found in proteins.**
- **The first to be discovered was asparagine, in 1806.**
- **Asparagine was first found in asparagus, and glutamate in wheat gluten; tyrosine was first isolated from cheese (Greek *tyros*, "cheese"); and glycine (Greek *glykos*, "sweet") was named because of its sweet taste.**
- **The 20 amino acids commonly found as residues in proteins contain an α -carboxyl group, an α-amino group, and a distinctive R group substituted on the α carbon atom.**



# What are enzymes?

- Enzymes are molecular catalysts referred as **Biocatalyst**.
- **Biocatalyst are** natural catalysts, that perform chemical transformations or biochemical reaction inside the living cells.
- Almost all enzymes are proteins
- Enzymes function by lowering transition-state energies and energetic intermediates and by raising the ground-state energy.
- Enzymes have molecular weights of several thousand to several million, yet can catalyze transformations on molecules as small as carbon dioxide and nitrogen.
- Their ability to catalyze reactions is attributable to their primary, secondary, tertiary, and quaternary structures.
- As catalysts, enzymes are highly specific and efficient for a particular chemical reaction.
- Cofactors are involved in reactions along with enzyme where molecules are oxidized, reduced, rearranged or connected.
- Enzymes can accelerate a given reaction up to million folds. For example carbonic anhydrase which catalyzes hydration of carbon di oxide can catalyze $10^6$ molecules per second
- Enzymes are highly specific and catalyze single or closely related reaction.

# ENZYMES- HISTORY



- Biological catalysis was first recognized and described in the late 1700s, in studies on the digestion of meat by secretions of the stomach.
- In the 1800s with examinations of the conversion of starch to sugar by saliva and various plant extracts.
- In, 1833, the first enzyme was isolated from aqueous extract of malt added ethanol.
- The heat-labile precipitate that is now known as amylase was utilized to hydrolyze starch to soluble sugar.
- In the 1850s, Louis Pasteur concluded that fermentation of sugar into alcohol by yeast is catalyzed by "ferments."
- He postulated that these ferments were inseparable from the structure of living yeast cells; this view, called vitalism, prevailed for decades.
- in 1897 Eduard Buchner discovered that yeast extracts could ferment sugar to alcohol, proving that fermentation was promoted by molecules that continued to function when removed from cells. Buchner's experiment at once marked the end of vitalistic notions and the dawn of the science of biochemistry.
- In 1878 Frederick W. Kühne, gave the name **enzymes** (from the Greek *enzymos*, "leavened") to the molecules detected by Buchner.

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ENZYMES- HISTORY

20-07-2020

- In 1898, Duclaux proposed that all enzymes should have suffix 'ase'.
- The isolation and crystallization of urease by James Sumner of Cornell University in 1926 was a breakthrough in early enzyme studies.
- Sumner postulated that all enzymes are proteins.
- He later, in 1946, received the Nobel Prize for his work with the enzyme urease.
- In the absence of other examples, this idea remained controversial for some time.
- Only in the 1930s was Sumner's conclusion widely accepted, after John Northrop and Moses Kunitz crystallized pepsin, trypsin, and other digestive enzymes and found them also to be proteins.
- Urease is an enzyme that catalyzes the conversion of urea to ammonia and carbon dioxide. Certain bacteria that convert urea to ammonia as part of the nitrogen cycle contain this enzyme.
- J. B. S. Haldane wrote a treatise titled *Enzymes*.

# NOMENCLATURE AND CLASSIFICATION



## INTRODUCTION

NC-IUBMB Enzyme List, or, to give it its full title, "Recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the Nomenclature and Classification of Enzymes by the Reactions they Catalyse, is a functional system, based solely on the substrates transformed and products formed by an enzyme.

## EC NUMBERS

enzymes are identified by EC (enzyme commission) numbers

These are also valuable for relating the information to other databases. They were divided into 6 major classes according to the type of reaction catalysed and a seventh, the translocases, was added in 2018. These are shown in Table 1

Reference: A Brief Guide to Enzyme Nomenclature and Classification Keith Tipton and Andrew McDonald)

## Table 1. Enzyme classes



| Sl. No. | Name | Reaction catalysed |
|---|---|---|
| 1 | Oxidoreductases* | *AH2 + B = A +BH2 |
| 2 | Transferases | AX + B = BX + A |
| 3 | Hydrolases | A-B + H2O = AH + BOH |
| 4 | Lyases | A=B + X-Y = AX-B Y |
| 5 | Isomerases | A = B |
| 6 | Ligases † | †A + B + NTP = A-B + NDP + P (or NMP + PP) |
| 7 | Translocases | AX + B\|\| = A + X + \|\| B (side 1) (side 2) |

*Where nicotinamide-adenine dinucleotides are the acceptors, NAD+ and NADH +

† NTP = nucleoside triphosphate.

# EC NUMBER



The EC number is made up of four components separated by full stops.

1. The first identifies the class of reaction catalysed.
   E.g. dehydrogenase (NADP+); EC 1.1.1.42:
   isocitrate + NADP+ = 2-oxoglutarate + CO2 + NADPH + H+
1. The second number (the subclass) generally contains information about the type of compound or group involved.
   e.g. For the oxidoreductases, the subclass indicates the type of group in the donor that undergoes oxidation or
   reduction (e.g., 1.1. acts on the CHOH group of donors whereas 1.4. acts on the CH-NH2 group of donors)
1. The third number, the sub-subclass, further specifies the type of reaction involved.
   For instance,
   EC 1.x.1.- indicates that NAD+ or NADP+ is the acceptor, while 1.x.2.- has a cytochrome as the acceptor, etc.
4. The fourth is a serial number that is used to identify the individual enzyme within a sub-subclass.

# NOMENCLATURE AND CLASSIFICATION



| CLASS | REACTION CATALYZED | TYPICAL REACTION | EXAMPLE(S) TRIVIAL NAME |
|---|---|---|---|
| EC 1 *Oxidoreductases* | To catalyze oxidation/reduction reactions; transfer of H and O atoms or electrons from one substance to another | AH + B → A + BH (reduced)<br>A + O → AO (oxidized) | Dehydrogenase, oxidase |
| EC 2 *Transferases* | Transfer of a functional group from one substance to another. The group may be methyl-, acyl-, amino- or phosphate group | AB + C → A + BC | Transaminase, kinase |
| EC 3 *Hydrolases* | Formation of two products from a substrate by hydrolysis | AB + $H_2O$ → AOH + BH | Lipase, amylase, peptidase, phosphatase |
| EC 4 *Lyases* | Non-hydrolytic addition or removal of groups from substrates. C-C, C-N, C-O or C-S bonds may be cleaved | RCOCOOH → RCOH + $CO_2$ or [X-A+B-Y] → [A=B + X-Y] | Decarboxylase |
| EC 5 *Isomerases* | Intramolecule rearrangement, i.e. isomerization changes within a single molecule | ABC → BCA | Isomerase, mutase |
| EC 6 *Ligases* | Join together two molecules by synthesis of new C-O, C-S, C-N or C-C bonds with simultaneous breakdown of ATP | X + Y + ATP → XY + ADP + $P_i$ | Synthetase |
| EC 7 *Translocases* | Catalyse the movement of ions or molecules across membranes or their separation within membranes | | Transporter |

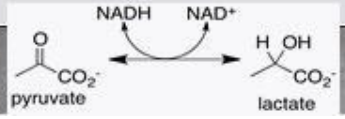
# NOMENCLATURE AND CLASSIFICATION



- **TRIVIAL NAME:** Except for some of the originally +studied enzymes such as pepsin, rennin, and trypsin, most enzyme names end in "ase". Example- Uricase (urate oxidase) catalyzes the following overall reaction:

  **Urate + $O_2$ + $H_2O$ ⟶ Allantoin + $H_2O_2$ + $CO_2$**

- ***SYSTEMATIC NAMES:*** The International Union of Biochemistry (I.U.B.) initiated standards of enzyme nomenclature which recommend that enzyme names indicate both the substrate acted upon and the type of reaction catalyzed.
- Under this system, the enzyme uricase is called urate: $O_2$ oxidoreductase
- This systematic name includes the name of the substrate or substrates in full and a word ending in '-ase' indicating the nature of the process catalysed.
- This word is either one of the six main classes of enzymes or a subdivision of one of them.
- When a reaction involves two types of overall change, e.g. oxidation and decarboxylation, the second function is indicated in brackets, e.g. oxidoreductase (decarboxylating). Example:
  (S)-lactate: NAD+ oxidoreductase (E.C. 1.1.1.27), trivial name lactate dehydrogenase, catalyses the reaction:



# MEASURES OF ENZYME ACTIVITY AND UNITS



- The enzyme unit was adopted by the International Union of Biochemistry in 1964
- The **enzyme unit**, or **international unit** for enzyme (symbol **U**, sometimes also **IU**) is a unit of enzyme's catalytic activity.
- 1 U (μmol/min) is defined as the amount of the enzyme that catalyzes the conversion of one μmol of substrate per minute under the specified conditions of the assay method
- Since the minute is not an SI base unit of time, the enzyme unit is discouraged in favor of the katal, the unit recommended by the General Conference on Weights and Measures in 1978 and officially adopted in 1999
- One katal is the enzyme activity that converts one mole of substrate per second under specified assay conditions, so
- 1 U = 1 μmol/min = 1/60 μmol/s ≈ 16.67 nmol/s;
- Therefore, 1 U = 16.67 nkat
- **Specific activity:** The specific activity of an enzyme is another common unit. This is the activity of an enzyme per milligram of total protein (expressed in μmol $min^{-1}$ $mg^{-1}$). Specific activity gives a measurement of enzyme purity in the mixture. It is the micro moles of product formed by an enzyme in a given amount of time (minutes) under given conditions per milligram of total proteins.



# GENERAL PROPERTIES OF ENZYMES



**1. HIGHER REACTION RATES:** The rates of enzymatically catalyzed reactions are typically factors of $10^6$ to $10^{12}$ greater than those of the corresponding uncatalyzed reactions and are at least several orders of magnitude greater than those of the corresponding chemically catalyzed reactions.

**2. MILDER REACTION CONDITIONS:** Enzymatically catalyzed reactions occur under relatively mild conditions: temperatures below 100°C, atmospheric pressure, and nearly neutral pH's. In contrast, efficient chemical catalysis often requires elevated temperatures and pressures as well as extremes of pH.

**3. GREATER REACTION SPECIFICITY:** Enzymes have a vastly greater degree of specificity with respect to the identities of both their **substrates** (reactants) and their products than do chemical catalysts; that is, enzymatic reactions rarely have side products. For example, in the enzymatic synthesis of proteins on ribosomes, polypeptides consisting of well over 1000 amino acid residues are made all but error free. Yet, in the chemical synthesis of polypeptides, side reactions and incomplete reactions presently limit the lengths of polypeptides that can be accurately produced in reasonable yields to about 200 residues .

**4. CAPACITY FOR CONTROL:** The catalytic activities of many enzymes vary in response to the concentrations of substances other than their substrates and products.The mechanisms of these control processes include allosteric control, covalent modification of enzymes, and variation of the amounts of enzymes synthesized.

# ENZYME ASSAYS



**TYPES OF ASSAY:** All enzyme assays measure either the consumption of substrate or production of product over time. Biochemists usually study enzyme-catalysed reactions using four types of experiments:

- **INITIAL RATE EXPERIMENTS:** When an enzyme is mixed with a large excess of the substrate, the enzyme-substrate intermediate builds up in a fast initial transient. Then the reaction achieves a steady-state kinetics in which enzyme substrate intermediates remains approximately constant over time Rates are measured for a short period after the attainment of the quasi-steady state, typically by monitoring the accumulation of product with time. The initial rate experiment is the simplest to perform and analyze. It is therefore by far the most commonly used type of experiment in enzyme kinetics.
- **PROGRESS CURVE EXPERIMENTS:** In these experiments, the kinetic parameters are determined from expressions for the species concentrations as a function of time. Progress curve experiments were widely used in the early period of enzyme kinetics, but are less common now.
- **TRANSIENT KINETICS EXPERIMENTS:** In these experiments, reaction behaviour is tracked during the initial fast transient as the intermediate reaches the steady-state kinetics period. These experiments are more difficult to perform than either of the above two classes because they require specialist techniques.
- **RELAXATION EXPERIMENTS:** In these experiments, an equilibrium mixture of enzyme, substrate and product is perturbed, for instance by a temperature, pressure or pH jump, and the return to equilibrium is monitored. The analysis of these experiments requires consideration of the fully reversible reaction.

# ENZYME ASSAYS



- Enzyme assays can be split into two groups according to their sampling method: **continuous assays**, where the assay gives a continuous reading of activity, and **discontinuous assays**, where samples are taken, the reaction stopped and then the concentration of substrates/products determined.
- **CONTINUOUS ASSAYS**

Continuous assays are most convenient, with one assay giving the rate of reaction with no further work necessary.

There are many different types of continuous assays.

- **Spectrophotometric**
- **Fluorometric**
- **Calorimetric**
- **Chemiluminescent**
- **Light scattering**
- **Microscale thermophoresis**
- **DISCONTINUOUS ASSAYS:**

Discontinuous assays are when samples are taken from an enzyme reaction at intervals and the amount of product production or substrate consumption is measured in these samples.

- **Radiometric**
- **Chromatographic**

# COENZYMES AND COFACTORS

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- **COFACTORS AND COENZYMES:** Some enzymes require no chemical groups for activity other than their amino acid residues. Others require an additional chemical component called a **cofactor**—either one or more inorganic ions, such as Fe, $Mg^{2+}$, $Mn^{2+}$, or $Zn^{2+}$, or a complex organic or metalloorganic molecule called a **coenzyme**.
- Coenzymes act as transient carriers of specific functional groups

**Holoenzyme = coenzyme and/or metal ions + apoenzyme or apoprotein**

TABLE 1: Some Inorganic Ions That Serve as Cofactors for Enzymes

| IONS | ENZYMES |
|---|---|
| $Cu^{2+}$ | Cytochrome oxidas |
| $Fe^{2+}$ or $Fe^{3+}$ | Cytochrome oxidase, catalase, peroxidase |
| K | Pyruvate kinase |
| $Mn^{2+}$ | Arginase, ribonucleotide reductase |
| $Mo^{2+}$ | Dinitrogenase |
| $Ni^{2+}$ | Urease |
| $Mg^{2+}$ | Hexokinase, glucose 6-phosphatase, pyruvate kinase |
| $Zn^{2+}$ | Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases |

TABLE 2: Some Coenzymes That Serve as Cofactors for Enzymes

| COENZYME | HOLOENZYME |
|---|---|
| NADPH | Malic enzyme, isocitrate dehydrogenase (IDH), and glutamate dehydrogenase |
| **Pyridoxal phosphate** | serine dehydratase and GDP-4-keto-6-deoxymannose-3-dehydratase (ColD) |
| **Biotin** | Acetyl-CoA carboxylase beta Methylcrotonyl-CoA carboxylase |
| Tetrahydrofolic acid | Formyltransferase |

A coenzyme or metal ion that is very tightly or even covalently bound to the enzyme protein is called a **prosthetic group**

# ENZYME ASSAYS

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- **DIRECT ENZYME ASSAY :**
- For some enzyme assays, it is possible to measure the reactant or product directly based on its absorbance properties Fersht (1999)
- In the reaction catalyzed by glucose-6-phosphate dehydrogenase (EC 1.1.1.49), one product (NADH) absorbs light at 340 nm, making it possible to monitor the reaction by following the increase in absorbance at this wavelength.

Glucose 6-phosphate+ $NAD^+$ → 6-phosphogluconate + NADH + $H^+$

- **COUPLED ENZYME ASSAYS:**
- Difficult detectable enzyme reactions are frequently coupled with easily observable reactions, preferentially NAD(P)H dependent dehydrogenases. An example is the hexokinase reaction connected with the glucose-6-phosphate dehydrogenase :

(1)Glucose+ATP→glucose-5-phosphate+ADP

(2)Glucose-6-phosphate+$NADP^+$→gluconate-6-phosphate+NADPH+$H^+$

The second, the *indicator* reaction can easily be detected by the absorption increase at 340 nm.

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