FE 361 Enzymology

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Content

• Enzyme structure
• Classification and Nomenclature of enzymes
• Enzyme unit
• Enzyme mechanism
• Factors affecting enzyme activity
ENZYMES

- **Enzymes** are proteins that accelerate chemical reactions.
- **Substrates**: The molecules present at the beginning of the reaction are called substrates.
- Enzymes convert substrates into different molecules, called products.

**Key definitions**

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Substance</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme</td>
<td>Protein</td>
<td>functions as biocatalysts</td>
</tr>
<tr>
<td>Substrate</td>
<td>Chemical compound</td>
<td>the enzyme work on</td>
</tr>
<tr>
<td>End product</td>
<td>Result</td>
<td>result of the reaction</td>
</tr>
</tbody>
</table>
Like all catalysts, enzymes work by lowering the activation energy for a reaction. Catalysts, like enzymes, act by lowering the energy difference between the reactants (A, B) and the transition state. This lowers the activation barrier for the reaction, allowing it to proceed more rapidly.

An uncatalyzed reaction requires a higher activation energy than does a catalyzed reaction. There is no difference in free energy between catalyzed and uncatalyzed reactions.
Nomenclature Of Enzymes

• Enzymes are usually named according to the reaction they carry out.
• Typically, the suffix ‘ase’ is added to the name of the substrate or the type of reaction (e.g. a polymerase or isomerase for a polymerization or isomerization reaction).
• Examples:
  • Maltose-maltase
  • Sucrose-sucrase
  • Lactose-lactase
  • Protein-protease
  • Lipids-lipase
  • Polymerization-polymerase
  • Isomerization-isomerase
• The exceptions to this rule are some of the enzymes studied originally, such as pepsin, rennin and trypsin.
2. The Nomenclature and Classification of Enzymes

- Various systems have been evolved to name and classify the enzymes over the period of times.

1. On the basis of substrate: naming the enzymes by adding the suffix \textit{–ase} in the name of substrate catalyzed. For example, enzymes acting upon carbohydrates were named as carbohydrases, upon lipids as lipases.

2. Enzymes nomenclature is also based upon the type of reaction catalyzed. Hydrolases (catalyzing hydrolysis), isomerases (isomerization).

3. Enzymes nomenclature based upon substrate and type of reaction catalyzed. For example, the enzyme succinic dehydrogenase catalyzes the dehydrogenation of the substrate succinic acid.

4. Enzyme nomenclature based upon substance that is synthesized. Fumarase that forms fumarate from malate.
2. The Nomenclature and Classification of Enzymes

• The International Enzyme Commission (EC) has recommended a systematic nomenclature for enzymes in addition to its existing trivial name to address the unambiguity and uniformity.

• This commission assigns names and numbers to enzymes according to the reaction they catalyze. An example of systematic enzyme name is EC 3.5.1.5 urea aminohydrolases for the enzyme that catalyzes the hydrolysis of urea.

• The name of an enzyme frequently provides a clue to its function. In some cases, an enzyme is named by incorporating the suffix -ase into the name of its substrate, e.g., pyruvate decarboxylases catalyzes the removal of a CO₂ group from pyruvate.

• Certain protein cutting digestive enzymes are exception to this general rule of enzyme nomenclature, e.g., pepsin, trypsin, chymotrypsin and thrombin.
EC Numbers

• Constitution of the four-digit EC number.

EC number: EC (i). (ii). (iii). (iv)

• (i) The main class, denotes the type of reaction it catalyzes

(ii) The subclass, indicates the type of substrate, type of transferred functional groups, the nature of specific bonds involved in the catalyzed reaction

(iii) Sub-sub class, indicates the nature of substrate or co-substrate

(iv) An arbitrary unique serial number

• Example: E.C. 2.7.1.1 represents class 2 (a transferase), subclass 7 (transfer of phosphate), sub-subclass 1 (an alcohol group as phosphate acceptor). The final digit denotes the enzyme, hexokinase or ATP: D-hexose-6-phosphotransferase. This enzyme catalyzes the transfer of phosphate from ATP to the hydroxyl group on carbon 6 of glucose.
Enzyme Classification Based on Reaction Types

Enzymes can be classified by the kind of chemical reaction catalyzed. Officially, six groups of enzymes have been classified:

<table>
<thead>
<tr>
<th>First digit</th>
<th>Enzyme class</th>
<th>Type of reaction catalysed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oxidoreductases</td>
<td>Oxidation/reduction reactions</td>
</tr>
<tr>
<td>2</td>
<td>Transferases</td>
<td>Transfer of an atom or group between two molecules (excluding reactions in other classes)</td>
</tr>
<tr>
<td>3</td>
<td>Hydrolases</td>
<td>Hydrolysis reactions</td>
</tr>
<tr>
<td>4</td>
<td>Lyases</td>
<td>Removal of a group from substrate (not by hydrolysis)</td>
</tr>
<tr>
<td>5</td>
<td>Isomerases</td>
<td>Isomerization reactions</td>
</tr>
<tr>
<td>6</td>
<td>Ligases</td>
<td>The synthetic joining of two molecules, coupled with the breakdown of pyrophosphate bond in a nucleoside triphosphate</td>
</tr>
</tbody>
</table>
The six main classes of enzymes

• EC 1: Oxidoreductases

involves redox reactions in which hydrogen or oxygen atoms or electrons are transferred between molecules.

\[ \beta\text{-D-glucose peroxide} + \text{oxygen} \rightarrow \text{D-glucono-1,5-lactone} + \text{hydrogen peroxide} \]
• **EC 2: Transferases** which catalyse the transfer of an atom or group of atoms (e.g., acyl-, alkyl- and glycosyl-), between two molecules, but excluding oxidoreductases and hydrolases. For example: aspartate aminotransferase (EC 2.6.1.1, systematic name, L-aspartate:2-oxoglutarate aminotransferase; also called glutamic-oxaloacetic transaminase or simply GOT).

\[
A-X + B \leftrightarrow BX + A
\]
• **EC 3: Hydrolases** catalyze hydrolytic reactions and their reversal. This is presently the most commonly encountered class of enzymes within the field of enzyme technology and includes the esterases, glycosidases, lipases and proteases. For example: chymosin (EC 3.4.23.4, no systematic name declared; also called rennin).

\[ A-X + H_2O \leftrightarrow X-OH + HA \]
EC 4: **Lyases** catalyze non-hydrolytic (covered in EC 3) removal of functional groups from substrates, often creating a double bond in the product; or the reverse reaction, ie, addition of function groups across a double bond. For example: histidine ammonia-lyase (EC 4.3.1.3, systematic name, L-histidine ammonia-lyase; also called histidase)

\[
A-B \rightarrow A=B + X-Y \\
X \ Y
\]
• **EC 5: Isomerases** catalyze isomerization reactions, including racemizations and cis-trans isomerizations. For example: xylose isomerase (EC 5.3.1.5, systematic name, D-xylose ketol-isomerase; commonly called glucose isomerase)

\[ \text{\(\alpha\)-D-glucopyranose} \quad \overset{\text{\(\rightarrow\)}}{=} \quad \text{\(\alpha\)-D-fructofuranose} \]
• **EC 6: Ligases**, also known as synthetases, catalyze the synthesis of various (mostly C-X) bonds, coupled with the breakdown of energy-containing substrates, usually ATP. For example: glutathione synthase (EC 6.3.2.3, systematic name, g-L-glutamyl-L-cysteine:glycine ligase (ADP-forming); also called glutathione synthetase).

\[
\text{ATP} + \gamma\text{-L-glutamyl-L-cysteine} + \text{glycine} \rightarrow \text{ADP} + \text{phosphate} + \text{glutathione}
\]
Chemistry of Enzymes

• Enzymes are generally globular proteins, having a size range from just 60 to more than 2500 amino acids.

• The activities of enzymes are determined by their three-dimensional structure.

• Most enzymes are much larger than the substrates they act on. It is therefore even more remarkable that only a small part of the enzyme molecule is directly involved in catalysis.

• This small section is called the active site and this site usually contains not more than a few (3–4) amino acids which are directly involved in the catalytic process. The substrate is normally bound by the enzyme in close proximity to, or even in, the active site.
An enzyme molecule has a depression called its **active site**, which is exactly the right shape for the substrate to fit into. The enzyme can be thought of as a **lock**, and the substrate as the **key**.
Specificity of Enzymes

• What is enzyme specificity?

  ▪ Ability of an enzyme to choose exact substrate
  ▪ It is a molecular recognition mechanism
  ▪ Recognition and specificity is based on structural complementarity

The specificity of enzymes is determined by complementary shape, charge, hydrophilic/hydrophobic characteristics of the substrates and their three-dimensional organization.
Specificity of Enzymes (Continued)

• One of the most relevant and also intriguing properties of enzymes is their specificity. In general, there are four distinct types of specificity:

(1) **Absolute specificity**: enzymes highly specific to one substrate and one reaction
Specificity of Enzymes (Continued)

(2) **Group specificity**: group specific enzymes act only on molecules that have specific functional groups, such as amino, phosphate or methyl groups.
(3) **Linkage specificity**: such enzymes act on chemical bonds of certain nature, regardless of the rest of the molecular structure.
(4) **Stereochemical specificity**: stereospecific enzymes act only on a particular steric or optical isomer and not on their isomeric counterparts.

**Stereo specificity of Enzymes**

- **α-1-4 Linked Glucose (Starch)**
  - **α-Amylase**

- **β-1-4 Linked Glucose (Cellulose)**
  - **α-Amylase**
Mechanisms

- Enzymes are very **specific** (fit to certain reaction only). To catalyse a reaction, enzyme molecule and substrate molecule need to meet and joint together by a temporary bond.
Enzymatic Reactions

Enzyme (E) combines with a substrate (S) to form new products (P):

\[ E + S \rightleftharpoons ES \rightarrow E + P \]
FACTORS AFFECTING ENZYME ACTIVITY

Several factors affect the rate at which reactions proceed, such as

- temperature,
- pH,
- enzyme concentration,
- substrate concentration and
- the presence of any inhibitors or activators.
- Ionic strength
**Effect of Temperature**

- The rate of an enzyme-catalyzed reaction increases as the temperature is raised.
- However, very **high** temperatures **denature** enzymes.
- Most animal enzymes rapidly become denatured at temperatures above 40°C.
- Enzymes are most active at their optimum temperature.
FACTORS AFFECTING ENZYME ACTIVITY (Continued)

- **Effect of pH**
  - The most favorable pH value - the point where the enzyme is most active - is known as the optimum pH.
  - Extremely high or low pH values generally result in complete loss of activity for most enzymes.
  - An extreme pH can **denature** enzymes – the active site is deformed permanently.
FACTORS AFFECTING ENZYME ACTIVITY (Continued)

• Effect of Substrate Concentration

• If the amount of the enzyme is kept constant and the substrate concentration is then gradually increased, the reaction velocity will increase until it reaches a maximum. After this point, increases in substrate concentration will not increase the velocity.
FACTORS AFFECTING ENZYME ACTIVITY (Continued)

- *Enzyme Concentration*

  - As the concentration of enzyme increases, rate of reaction will also increase (at constant substrate concentration)
For centuries, enzymes have been employed in a variety of applications such as beer and cheese production.

Industrial enzymes may be derived from a wide variety of plant, animal or microbial sources, although most production processes rely on microbial sources. However, over the years, advancements in biotechnology have resulted in newer and more highly efficient varieties of enzymes.

The industrial success of enzymes can be attributed to certain key benefits that enzymes offer in comparison with chemicals.

- The combination of catalytic function,
- specificity and
- the ability to work under reasonably mild conditions

makes enzymes the preferred catalyst in a variety of applications.
INDUSTRIAL ENZYMES (Continued)
Industrial enzymes are prepared and commercialized as partly purified or ‘bulk’ enzymes, as opposed to highly purified enzymes for analytical or diagnostic use.

Industrial enzymes represent the heart of biotechnology. Advancements in biotechnology and genomics have aided the discovery of fresh enzyme sources and production strains for commercialization. With advances in biotechnology, the horizon of enzyme applications is getting broader day by day.

Enzymes can be used not only for chemical processes, but also for mechanical and physical processes.
INDUSTRIAL ENZYMES (Continued)

• Microbes are preferred to plants and animal as industrial enzyme source for the following reasons:

1. Higher expression levels.

2. Higher purity (% enzyme protein vs. % other components).

3. Cheaper production due to the above.

4. Their enzyme contents are more predictable and controllable

5. Plant and animal tissues contain more potentially harmful materials than microbes
In industry, generally immobilized enzymes are used.

Immobilized enzyme is either physically entrapped or covalently bonded by chemical means to an inert insoluble matrix or carrier.

Advantages of Immobilized Enzymes:

- Attachment to polymers/matrix, causes re-use
- Cost effective
- Not difficult to separate
- Potential in industrial and medicinal uses
- Stability of the enzyme increases
Enzyme Applications in Food Industry

• In food production, enzymes have a number of advantages.

(1) Enzymes are alternatives to traditional chemical-based technology. Enzymes can thus replace synthetic chemical-based technology.

(2) Environmental performance of processes by lowering energy consumption

(3) Enzymatic processes have fewer side reactions and by-products (waste-products) since they are more specific.

The result is higher quality products and less pollution.

(4) Enzymes can catalyze reactions under very mild conditions, allowing mild processing conditions which do not destroy valuable attributes of foods and food components.
Enzyme Applications in Food Industry (Continued)

- **ENZYMES USED IN STARCH INDUSTRY**
- Production of high fructose corn syrup and sweeteners

<table>
<thead>
<tr>
<th>FEEDSTOCK</th>
<th>PRODUCT</th>
<th>ENZYME(S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STARCH</td>
<td>CORN SYRUPS</td>
<td>α-AMYLASES, PULLULANASE</td>
</tr>
<tr>
<td></td>
<td>GLUCOSE</td>
<td>α-AMYLASE, GLUCOAMYLASE</td>
</tr>
<tr>
<td></td>
<td>FRUCTOSE</td>
<td>α-AMYLASE, GLUCOAMYLASE, GLUCOISOMERASE</td>
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</tbody>
</table>
Enzyme Applications in Food Industry (Continued)

- **ENZYMES IN MILK AND DAIRY PRODUCTS**

<table>
<thead>
<tr>
<th>ENZYMES</th>
<th>FUNCTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chymosin</td>
<td>Milk coagulation</td>
</tr>
<tr>
<td>Proteases</td>
<td>Flavour improvement, decrease ripening time of cheeses</td>
</tr>
<tr>
<td>Lipases</td>
<td>Flavour improvement, decrease ripening time of cheeses</td>
</tr>
<tr>
<td>Sulphydryl oxidase</td>
<td>Remove cooked flavour</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>Lactose removal</td>
</tr>
<tr>
<td>Proteases</td>
<td>Soyabean milk coagulation</td>
</tr>
</tbody>
</table>
## Enzyme Applications in Food Industry (Continued)

### ENZYMES IN BAKING

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase</td>
<td>Maximise fermentation purpose; prevent staling</td>
</tr>
<tr>
<td>Protease</td>
<td>Improve handling and rheological properties</td>
</tr>
<tr>
<td>Glutamyl transferase</td>
<td>Improve dough elasticity; retention of bread softness on storage</td>
</tr>
</tbody>
</table>
Enzyme Applications in Food Industry (Continued)

- ENZYMES IN BREWING

<table>
<thead>
<tr>
<th>ENZYME</th>
<th>PURPOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>amylase(α and β)</td>
<td>Convert starch to maltose and dextrins</td>
</tr>
<tr>
<td>Proteases</td>
<td>Hydrolyze proteins to amino acids; used by yeast to grow</td>
</tr>
<tr>
<td>Papain</td>
<td>Chill proofing beer</td>
</tr>
</tbody>
</table>