Enzymes

• Enzymes are biological catalysts. They increase the rate at which equilibrium is reached, but they do not affect the equilibrium.

• Enzymes differ from ordinary chemical catalysts:
  ⇒ Enzyme catalyzed reaction rates are typically $10^6$ to $10^{12}$ greater than those of the uncatalyzed reactions.
  ⇒ Enzyme catalyzed reactions occur under relatively mild conditions (physiological conditions).
  ⇒ Enzymes often have tremendous specificity with regards to both their substrates and products.
  ⇒ The activity of enzymes can be regulated by compounds other than their substrates and products.
Classes of Enzymes

<table>
<thead>
<tr>
<th>Classification</th>
<th>Type of Reaction Catalyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Oxidoreductases</td>
<td>Oxidation–reduction reactions</td>
</tr>
<tr>
<td>2. Transferases</td>
<td>Transfer of functional groups</td>
</tr>
<tr>
<td>3. Hydrolases</td>
<td>Hydrolysis reactions</td>
</tr>
<tr>
<td>4. Lyases</td>
<td>Group elimination to form double bonds</td>
</tr>
<tr>
<td>5. Isomerases</td>
<td>Isomerization</td>
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<tr>
<td>6. Ligases</td>
<td>Bond formation coupled with ATP hydrolysis</td>
</tr>
</tbody>
</table>

Hydrolases and oxidoreductases are of particular interest.

Enzymes in Organic Solvents

- Enzymes have evolved to function in an aqueous environment.
- This has hampered their use in organic synthesis.
- Over the last 15 years it has been found that enzymes can also function in nonaqueous environments.
- Enzyme properties are affected by nonaqueous conditions and may differ significantly from those normally associated with the enzyme in an aqueous environment.
Enzymes in Organic Solvents

- Many enzymes demonstrate increased thermal stability in organic media.
- Lipase in nonaqueous conditions has been determined to be stable at 100°C for several hours. This is not true for the enzyme in aqueous solution.
- It also demonstrated higher activity at 20°C than lipase in aqueous solution.
- This enhanced stability decreases as the water content increases.

Enzymes in Organic Solvents

Various approaches for using enzymes in organic solvents:

A. Enzyme solvated in the normal aqueous environment.
B. Enzymes in a low-water solvent system. Enzyme is suspended in an organic solvent.
C. A microemulsion where the enzyme is encapsulated within a reverse micelle.
D. Monophasic cosolvent system where the enzyme is dissolved in a mixture of water and water miscible organic solvents.
E. A biphasic organic-aqueous system where the enzyme resides in the aqueous layer.
Enzymes in a Low-Water Solvent System

• In organic media, enzymes require water for catalytic activity.
• In general, enzymatic catalysis improves with increased hydration (referring to water associated with protein, not water content of system as a whole).
• The degree of hydration required depends on the enzyme and the organic solvent.
  ⇒ Lipases appear to require very few water molecules in order to be active.
  ⇒ Some enzymes (such as chymotrypsin) are active when associated with 50 or fewer water molecules.
  ⇒ Others (such as alcohol dehydrogenase) require sufficient water molecules for a monolayer to form.

Enzymes in a Low-Water Solvent System

• Under anhydrous conditions, the charged groups on the protein surface may interact tightly with each other, resulting in an inactive “locked” conformation.
• The presence of water molecules may increase enzyme flexibility allowing the enzyme to be catalytically active.
• The nature of the organic solvent also influences the behavior of suspended enzymes.
  ⇒ Enzyme activity
  ⇒ Substrate specificity
  ⇒ Regioselectivity
  ⇒ Enantioselectivity
  ⇒ Prochiral selectivity
Enzymes in a Low-Water Solvent System

- Polar organic solvents are capable of stripping the hydrating water molecules from the protein surface, decreasing enzyme activity.
- Hydrophobic solvents are less likely to disrupt or distort the aqueous layer surrounding the enzyme, promoting increased enzyme activity.
- Solvent may also affect enzyme activity by direct interaction with the substrates or products.
- The solvent may affect the effective substrate or product concentrations (particularly important in enzymes prone to substrate or product inhibition.

Enzymes in Organic Solvents

- Enzymes are generally insoluble in organic solvents (DMSO being an exception) allowing for easy recovery of enzyme after use.
- One approach to nonaqueous biocatalysis is to utilize the enzyme as a fine powder suspended in the organic solvent.
- Another approach is to immobilize the enzyme by adsorbing it onto an inert support such as celite or porous silica.
- **Enzyme preparation, can affect enzyme properties and activity.**
Suspended Enzyme

- Enzyme powders suspended in organic solvents tend to be more rigid than when in an aqueous solution.
- It has been proposed that the unique properties associated with enzymes in a nonaqueous environment is due in large part to this increased rigidity.
- The enzymes used in organic solvents are generally either lyopholyzed or precipitated from an aqueous solution with a pH optimal for enzymatic activity.
- In the solid powder form, the ionogenic groups of the enzymes appear to retain the ionization state associated with the pH of the parent aqueous solution.
- The pH associated with optimal performance in the organic suspension may differ from that of the enzyme in aqueous conditions. This shows some dependence on the organic solvent.

<table>
<thead>
<tr>
<th>pH</th>
<th>Aqueous Solution</th>
<th>Nonaqueous Solution (heptane)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td></td>
<td>maximum activity</td>
</tr>
<tr>
<td>7.5</td>
<td></td>
<td>maximum activity</td>
</tr>
<tr>
<td>11.0</td>
<td></td>
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</table>
Adsorbed Enzyme

- Selection of support material is important.
- The support (depending on its hydrophobicity) can influence the effective substrate/product concentration in the vicinity of the enzyme active site.
- The support can also affect enzyme hydration. Enzymes adsorbed on a hydrophobic support demonstrate increased activity due to improved hydration.
- The support can also affect the kinetics of an enzyme-catalyzed reaction.
- The porosity of the support can affect substrate/product diffusion and degree of enzyme loading on the solid support.

Enzymes in Organic Solvents

*Hydrolases*

- Under nonaqueous conditions hydrolases (lipases and proteases) catalyze esterification, transesterification and amide condensation reactions rather than hydrolytic reactions.
- In a water-restricted environment the equilibrium of amide hydrolysis is shifted towards amide bond formation.
- Demonstrated in the enzymatic synthesis of the dipeptide L-Asp-L-Phe-OCH₃ (aspartame) using thermolysin.
Enzymes in Organic Solvents

**Hydrolases**

- Use of hydrolases in organic solvents allows the enzymatic synthesis of polymers such as polyesters and polyacrylates.
- This is not possible under aqueous conditions due to low solubility of monomers and/or the polymer product.
- Use of enzymes to fabricate polymers capitalizes on the selectivity associated with enzyme catalyzed reactions.
- Enzymes can be used in conjunction with traditional catalysts for chemo-enzymatic synthesis of polymers.

**Oxidoreductases**

- As in the case of hydrolases, the catalytic activities of oxidoreductases are affected by a nonaqueous environment.
- Most oxidoreductases require cofactors in order to carry out redox reactions.
- In order to function properly, it is necessary to regenerate the cofactor.
- YADH in heptane efficiently oxidizes 3-methyl-2-buten-1-ol to the corresponding aldehyde.
- The cofactor (NAD+) can be recycled *in situ* by the addition of acetone.
Enzymes in Organic Solvents

- Attachment of amphiphilic polymers such as PEG to enzymes can be used to generate enzyme constructs that are soluble in organic solvents. (PEG-modified enzymes are soluble in organic solvents in the mg/ml range.)
- The specific activities of such PEG-enzyme constructs are often similar to those of the free enzyme under aqueous conditions.
- The PEG-modified enzymes generally demonstrate greater stability than the parent enzymes.
- May also see changes in the optimal temperature for enzyme activity.

⇒ PEG-lipase catalyzing ester exchange reaction under nonaqueous conditions ---> optimal temp. 70°C
⇒ Lipase catalyzing the hydrolysis of esters under aqueous conditions ---> optimal temp. 45°C.

PEGylation of enzymes using a 2,4-bis(mPEG)-6-s-chlorotriazine