CHAPTER 9

CATALYTIC STRATEGIES USED BY ENZYMES

1. Covalent catalysis
   (reactive group in active site)

2. General acid-base catalysis
   (proton donor or acceptor)

3. Metal ion catalysis
   (metal ion involved in catalysis)

4. Catalysis by approximation
   (two distinct substrates bind to active site)
CLASSES OF PROTEASES

1. Serine proteases (e.g. chymotrypsin)

2. Cysteine (or thiol or sulphhydryl) proteases (e.g. papain)

3. Aspartyl (or carboxyl or acid) proteases (e.g. pepsin)

4. Metallloproteases (or zinc proteases) (e.g. carboxypeptidase A)
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypsin</td>
<td>Pancreas</td>
<td>Digestion of proteins</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>Pancreas</td>
<td>Digestion of proteins</td>
</tr>
<tr>
<td>Elastase</td>
<td>Pancreas</td>
<td>Digestion of proteins</td>
</tr>
<tr>
<td>Thrombin</td>
<td>Vertebrate serum</td>
<td>Blood clotting</td>
</tr>
<tr>
<td>Plasmin</td>
<td>Vertebrate serum</td>
<td>Dissolution of blood clots</td>
</tr>
<tr>
<td>Kallikrein</td>
<td>Blood and tissues</td>
<td>Control of blood flow</td>
</tr>
<tr>
<td>Complement C1</td>
<td>Serum</td>
<td>Cell lysis in the immune response</td>
</tr>
<tr>
<td>Acrosomal protease</td>
<td>Sperm acrosome</td>
<td>Penetration of ovum</td>
</tr>
<tr>
<td>Lysosomal protease</td>
<td>Animal cells</td>
<td>Cell protein turnover</td>
</tr>
<tr>
<td>Cocoonase</td>
<td>Moth larvae</td>
<td>Dissolution of cocoon after metamorphosis</td>
</tr>
<tr>
<td>α-Lytic protease</td>
<td><em>Bacillus sorangium</em></td>
<td>Possibly digestion</td>
</tr>
<tr>
<td>Proteases A and B</td>
<td><em>Streptomyces griseus</em></td>
<td>Possibly digestion</td>
</tr>
<tr>
<td>Subtilisin</td>
<td><em>Bacillus subtilis</em></td>
<td>Possibly digestion</td>
</tr>
</tbody>
</table>

CHYMOTRYPSIN

Main features:

- Mammalian digestive enzyme
- It hydrolyzes peptide bonds on the carboxyl side of aromatic and hydrophobic residues, such as methionine, and ester bonds
- Member of the serine protease family
- 25 kDa protein
- It consists of three polypeptide chains connected by two interchain disulfide bonds
- Dimensions: 51 x 40 x 40 Å
- Secondary structure: mainly antiparallel β-pleated sheet, little α helix
Figure 11-30. The activation of chymotrypsinogen by proteolytic cleavage. Both \( \pi \)- and \( \alpha \)-chymotrypsin are enzymatically active. See Kinemage Exercise 10-4.
Figure 9-3
$p$-Nitrophenol liberated

Burst phase

Steady-state phase

Millisconds after mixing

Enzyme + $\text{C-CH}_3$ $\rightarrow$ Enzyme + $\text{p-Nitrophenol}$

$\text{C-CH}_3$

$\text{O}$

$\text{NO}_2$

$p$-Nitrophenyl acetate

Acetyl-enzyme intermediate $p$-Nitrophenol

Enzyme + $\text{H}_2\text{O}$ $\rightarrow$ Enzyme + $\text{Acetate} + \text{H}^+$

Acetyl-enzyme intermediate

Acetate

Figures 9-29, 9-30, and 9-31, page 223

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Figure 9-5
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It is synthesized as a single-chain inactive precursor called chymotrypsinogen.

Catalytic triad:

Serine 195, Histidine 57, Aspartate 102

Steps of the Catalytic Mechanism:

1. Hydrolysis of the peptide bond starts with an attack by the oxygen atom of the hydroxyl group of Ser-195 on the carbonyl carbon atom of the susceptible peptide bond.

2. Formation of a transient tetrahedral intermediate.

3. Transfer of a proton from Ser-195 to His-57.

4. Deacylation.
Trypsin & Elastase:

Trypsin and elastase are like chymotrypsin in many respects:

- About 40% of the amino acid sequences of these three enzymes are identical

- Their 3-D structures are very similar

- A serine-histidine-aspartate catalytic triad is present in all three

- The serine residue of the catalytic triad is modified by fluorophosphates

- The amino acid sequence around this serine is the same in all three enzymes: Gly- Asp- Ser- Gly- Gly-Pro

- All three enzymes have identical catalytic mechanisms
Chymotrypsin
Specificity: Phe, Trp, Tyr
Met, His, Asn, Leu

Trypsin
Asp
Specificity: (Ser → Asp)
Lys, Arg

Elastase
Specificity: (Gly → Val)
Ala, Gly, Ser, Val

Figures 9-39 and 9-40, page 227
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FIGURE 14-22. A diagram indicating the relative positions of the active site residues in the primary structures of subtilisin (left), chymotrypsin (middle), and serine carboxypeptidase II (right). The catalytic triad consists of Ser 221, His 64, and Asp 32 in subtilisin and of Ser 146, His 397, and Asp 338 in serine carboxypeptidase II. The peptide backbones of Ser 214, Trp 215, and Gly 216 in chymotrypsin, and their counterparts in subtilisin, participate in substrate-binding interactions. [After Robertus, J.D., Alden, R.A., Birktoft, J.J., Kraut, J., Powers, J.C., and Wilcox, P.E., Biochemistry 11, 2449 (1972).]
Pepsin:

- Aspartyl protease
- 35-kDa- single- chain protein
- Optimum pH for activity: 2- 3
- The active site contains a water molecule flanked by two aspartates
- It consists of two structurally similar lobes

The two aspartates, one on each lobe, play a dual role in catalysis:

1. They activate the water molecule positioned between them
2. They serve as protons acceptors and donors
Figure 9-44, page 229; Figures 9-45 and 9-46, page 230

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CHAPTER 9 (Proteases)

Problems

- From textbook
  
  # 1, 2, 3, 4

- From companion
  
  # 2, 9, 10, 12