Enzyme Catalytic Mechanisms

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Serine Proteases

- Cleave Peptide Bonds
- Specificity of Cutting
- Common Active Site Composition/Structure
- Mechanistically Well Studied

Chymotrypsin
Chymotrypsin Catalysis
Chymotrypsin

N-Acetyl-L-phenylalanine p-nitrophenyl ester

Yellow Color
Serine Proteases

Color Produced

Burst phase

Time (seconds)
Serine Proteases

- Catalytic Mechanism

Substrate for Enzyme

Folded Polypeptide Chain of Enzyme

Catalytic Triad of Active Site
Serine Proteases
• Catalytic Mechanism

Region of Enzyme That Determines What Substrate the Enzyme Binds
Serine Proteases
• Catalytic Mechanism

1. Binding of Substrate Stimulates Slight Structural Changes

2. Structural Changes Induced by Binding Change Electronic Environment of Catalytic Triad

[Diagram showing the catalytic triad with Ser, His, and Asp residues and the S1 pocket of the enzyme]
Serine Proteases

- Catalytic Mechanism

N abstracts Proton From Serine’s Side Chain, Creating Alkoxide Ion
Serine Proteases
• Catalytic Mechanism

Alkoxide Ion Makes Nucleophilic Attack on Carbonyl Carbon of Peptide Bond
Serine Proteases

- Catalytic Mechanism

Peptide Bond Broken as N Binds to H on Histidine

Tetrahedral Intermediate Stabilized by Oxyanion Hole
Serine Proteases

- Catalytic Mechanism

Half of Polypeptide Released from Enzyme

Other Half of Polypeptide Covalently Linked to Serine

Fast Phase of Catalysis Completed

Enzyme Backbone

His

Asp

Enzyme Backbone

Released

\[ \text{Ser} \]

\[ \text{O} \]

\[ \text{O} \]

\[ \text{C} \]

\[ \text{R} \]

\[ \text{HN} \]

\[ \text{R'} \]
Serine Proteases

- Catalytic Mechanism

1. Water Enters Active Site

2. Nitrogen Attacks Proton of Water

3. Hydroxide Attacks Carbonyl Carbon
Serine Proteases
• Catalytic Mechanism

Oxyanion Hole Stabilizes Tetrahedral Intermediate

Oxygen Abstracts H on N-Group, Breaking Bond with Serine
Serine Proteases

• Catalytic Mechanism

Slow Phase of Catalysis Complete
Serine Proteases
• Catalytic Mechanism

Other Polypeptide Fragment Released

Enzyme Returned to Original State

Cycle Complete. Enzyme Ready to Start Anew
Serine Proteases

- Catalytic Mechanism

1. Binding of substrate to S1 pocket
2. Formation of alkoxide ion
3. Nucleophilic attack
   Stabilization of intermediate
4. Breakage of peptide bond
5. Release of peptide 1
6. Entry and activation of water
7. Release of peptide 2 from enzyme
S1 Pockets and Specificities

- Lysine
- Arginine
- Phenylalanine
- Tryptophan
- Tyrosine
- Glycine
- Alanine
- Valine
Serine Proteases

- Site-directed mutagenesis
  - No mutation - activity = 100
  - Serine to alanine - activity = 0.00001
  - Histidine to alanine - activity = 0.00001
  - Aspartic acid to alanine - activity = 0.001
  - All three to alanine - activity = 0.00001
  - No enzyme - activity = 0.000000001
Protein Cleavage Agents

**Subtilisin** - C-terminal side of large uncharged side chains

**Chymotrypsin** - C terminal side of aromatics (Phe, Tyr, Trp)

**Trypsin** - C-terminal side of lysine and arginines (not next to proline)

**Carboxypeptidase** - N-terminal side of C-terminal amino acid

**Elastase** - Hydrolyzes C-side of small AAs (Gly, Ala)

**Cyanogen Bromide** (chemical) - Hydrolyzes C-side of Met

NH₃-Cysteine-Arginine-Methionine-Glycine-Phenylalanine-Aspartic Acid-Leucine-COOH
Inhibiting Protease Action

Serpins = Serine Protease Inhibitors

α-1-antitrypsin
Reactive oxygen species

A missing electron creates a "Free Radical", highly reactive

\[
\begin{align*}
\text{H}_3\text{C-S-} & \text{CH}_2-\text{COOH} \\
\text{H}_3\text{C-S-} & \text{CH}_2-\text{NH}_2
\end{align*}
\]

\[
\begin{align*}
\text{H}_3\text{C-S-} & \text{CH}_2-\text{COOH} \\
\text{H}_3\text{C-S-} & \text{CH}_2-\text{NH}_2
\end{align*}
\]
Prevalence of $\alpha$-1-antitrypsin deficiency

$\alpha$-1-antitrypsin deficiency by %
The New Serine Protease Song
(to the tune of “Rudolph the Red-Nosed Reindeer”)
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Metabolic Melody

(Intro)
All serine proteases
Work almost identically
Using amino acid Triads catalytically

First they bind peptide substrates
Holding onto them so tight
Changing their structure when they
Get them in the S1 site

Then there are electron shifts
At the active site
Serine gives up its proton
As the RE-ac-tion goes on

Next the alkoxide ion
Being so electron rich
Grabs peptide’s carbonyl group
Breaks its bond without a hitch

So one piece is bound to it
The other gets set free
Water has to act next to
Let the final fragment loose

Then it’s back where it started
Waiting for a peptide chain
That it can bind itself to
Go and start all o’er again