Lectures overview

- Intro + characteristics
- Kinetics
- Regulation
- Mechanisms
Multiple substrate/product reactions

- ENZYME reactions have binding and catalytic steps

\[ E + S \rightleftharpoons ES \rightarrow E + P \]

\[ E + S \rightleftharpoons ES \rightarrow E + P \]

\[ k_1 \quad k_{\text{cat}} \]

\[ k_{-1} \]

Still \textit{Michaelis-Menten} kinetics?
Multiple substrate/product reactions

\[ A + B \overset{\text{E}}{\rightleftharpoons} C + D \]

1) Sequential method

\[ E + A + B \rightarrow EAB \rightarrow ECD \rightarrow E + C + D \]

May be ordered or random
Multiple substrate/product reactions

A + B ⇌ C + D

2) Ping-pong method

E + A → EA → E-C → E' → E-B → ED → E+D

B

C

Covalent bonds to E formed during process

E + S → ES → EP → E + P
Activation energy

Binding energy partially offsets activation energy
Enzyme strategies

General strategies

a) Position the reactants correctly for interaction
b) Distort the reactants making them less stable
c) Stabilise transition state (TS)
d) Change the environment to favour the reaction
Enzyme strategies
General strategies

a) Only one angle of approach
b) Destabilisation of substrate
c) Stabilisation of the transition state

Affinity: Transition > Substrate > Product

Best inhibitor drugs resemble transition state

May bind *8 orders of magnitude* more strongly than S
Enzyme strategies

General strategies

d) Provision of enclosed chemical environment where *e.g.* pH can differ from outside

*e.g.* Tryptophan synthase in *Salmonella*
Enzyme strategies
General strategies

1) Covalent catalysis - a.s.r reacts with substrate

2) Acid/base catalysis - a.s.r accepts/donates H⁺ (Basic AA, Acidic AA, Cys, Ser, Tyr)

3) Metal ion catalysis - various types

Combination of non-covalent binding effects and covalent chemical interactions lowers overall AE
Peptide bonds can be cleaved (hydrolysed) by:

- Boiling in 6M hydrochloric acid for 24 hours

→Peptide bonds are very stable
Serine proteases

Over 18,000 serine proteases. Grouped into 12 clans and 40 families.

- Gene duplication and divergence
  - Trypsin
  - Chymotrypsin
  - Elastase
  - Thrombin

- Same reaction (hydrolysis of peptide bond)
- Different substrates (binding specificity)
Serine proteases

Chymotrypsin

Trypsin

Elastase

Thrombin

Large Hydrophobic
    e.g. Phe

Lys, Arg

Small Neutral
    Ala, Ser

Arg-Gly
Serine proteases

Specificity pocket

Chymotrypsin

Asp 189

Trypsin

Val 216

Elastase

Val 190
Serine proteases

Chymotrypsin active site

SP lined by hydrophobic residues
Serine proteases

Trypsin active site

Positively charged side-chains bind
Serine proteases

Elastase active site

Only small side chains can enter
Serine proteases
Reaction mechanism

Chymotrypsin

Catalytic triad (D/H/S)
Serine proteases
Reaction mechanism
Serine proteases
Reaction mechanism

- Formation of a *nucleophile* by catalytic triad

- His removes H\(^+\) from Ser (acid/base catalysis)
- Ser becomes a strong nucleophile which reacts with substrate (covalent catalysis)
- DIPF reacts with Ser195 (but not other Ser)
- TPCK reacts with His57 (but not other His)
Serine proteases
Reaction mechanism

- Step 1 - Nucleophilic attack on polypeptide carbonyl
Serine proteases

Reaction mechanism

- Step 2 - Covalent intermediate

Diagram showing the reaction mechanism with labels for Asp, His, Ser, and a tetrahedral intermediate.
Serine proteases
Reaction mechanism

- Step 3 - Cleavage and loss of *C-terminal fragment*
Serine proteases

Reaction mechanism

• Step 4 – Nucleophilic attack on polypeptide carbonyl by *water*

(His removes H from water [acid/base catalysis] to make OH⁻ nucleophile)
Serine proteases

Reaction mechanism

- Step 5 - Covalent intermediate
Serine proteases

Reaction mechanism

- Step 6 - Cleavage and loss of *N-terminal fragment*
Serine proteases

Oxyanion hole

H-bonds with NH on Gly93 and Ser195 secure O⁻
Other proteases

Nucleophilic attack on peptide bond

Cysteine proteases

Aspartyl proteases (e.g. pepsin)

Metalloproteases
Summary

- >1 substrate molecule $\rightarrow$ different routes
- AE is reduced by a combination of strategies
- Serine proteases use covalent and acid/base catalysis + destabilisation of substrate and TS stabilisation
- Same reaction mechanism used in many enzymes
- Substrate determined by binding residues