Factors that Affect Enzyme Catalysis

Ionic strength: $\mu = \frac{1}{2} \Sigma(Z_i^2 c_i)$

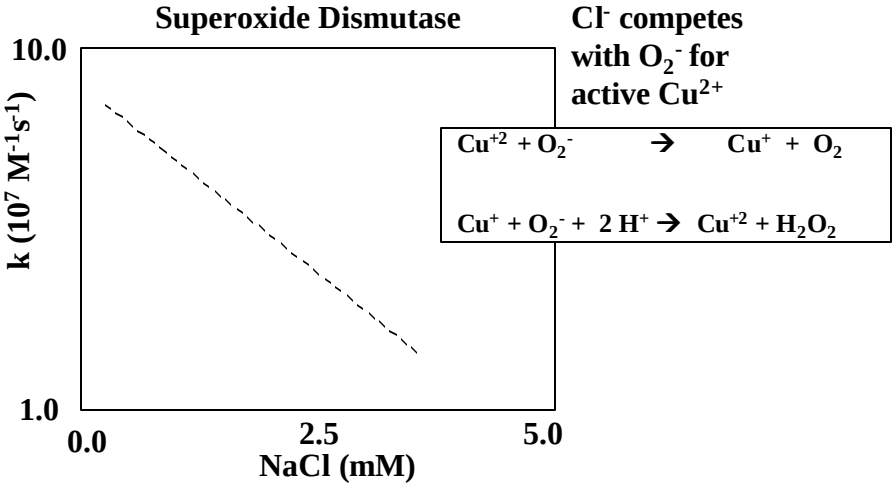
Review: urea, guanidinium hydrochloride, detergents, or organic solvents

Temperature

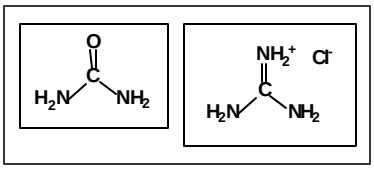
pH

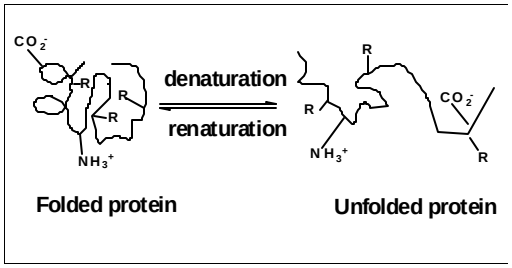
Factors that Affect Enzyme Catalysis: μ

Ionic strength: $\mu = \frac{1}{2} \Sigma(Z_i^2 c_i)$



Conditions that cause protein denaturation: Urea/Guandinium·HCl



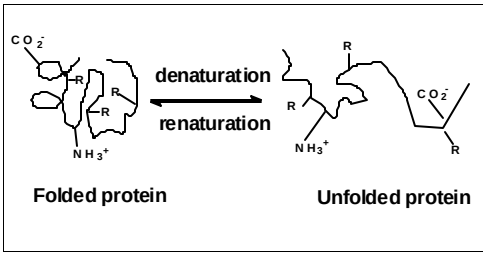


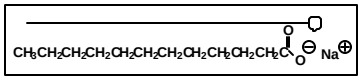
-Addition of urea or guanidinium hydrochloride (G·HCl)

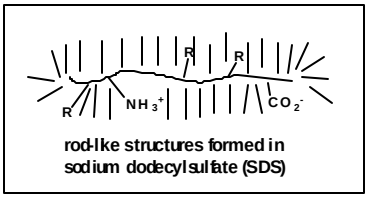
-Urea and G·HCl both H-bond to proteins causing disruption of the H-bond stabilization.

-Proteins unfold and remain soluble (usually).

Conditions that cause protein denaturation: Soaps/Detergents





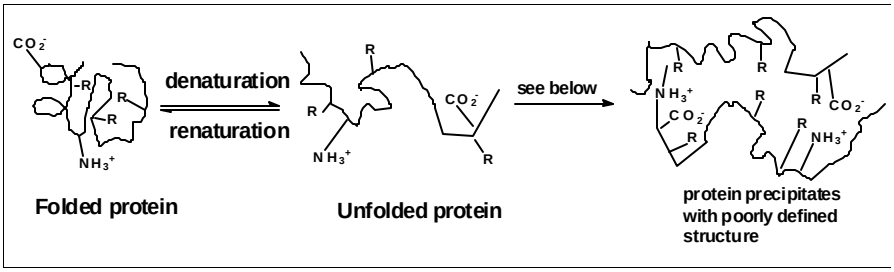


<u>Addition of soaps or detergents</u>

Nonpolar portions of soaps and detergents interact with protein R groups causing the loss of the hydrophobic stabilization.

Proteins denature and remain soluble.

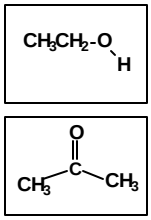
Conditions that cause protein denaturation: Organic Solvents



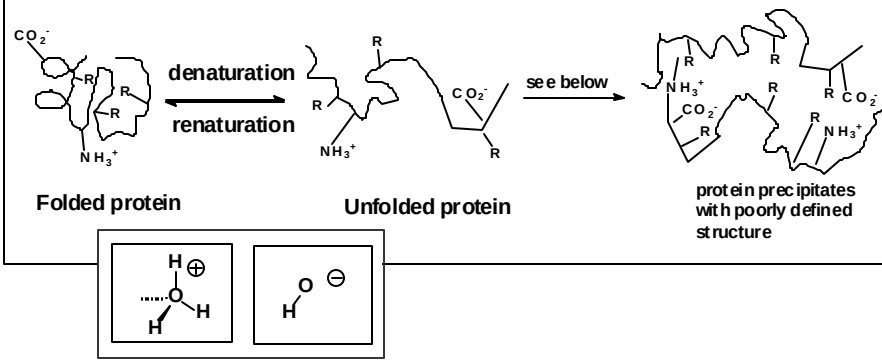
Addition of water-soluble organic solvents

Solvent molecules interact with protein R groups causing the loss of the hydrophobic stabilization.

Proteins denature and often precipitate.



Conditions that cause protein denaturation: H^+/OH^-



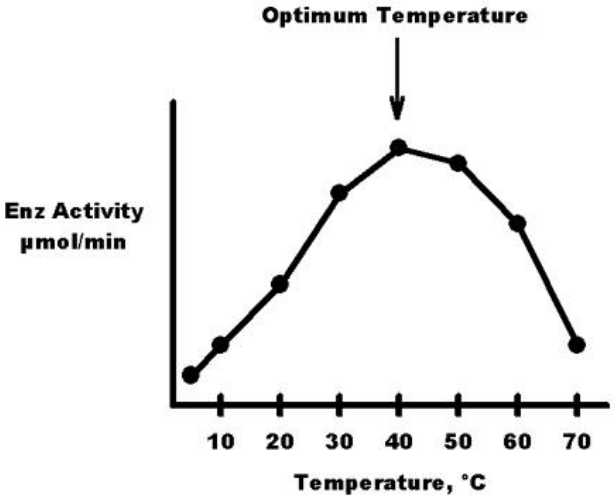
Extremes of pH

-OH^- and H_3O^+ both H-bond to proteins causing disruption of the H-bond stabilization.

-Some proteins may unfold and aggregate into denatured precipitates (solids). This is more common at low pHs.

Factors that Affect Enzyme Catalysis: Temperature

Temperature



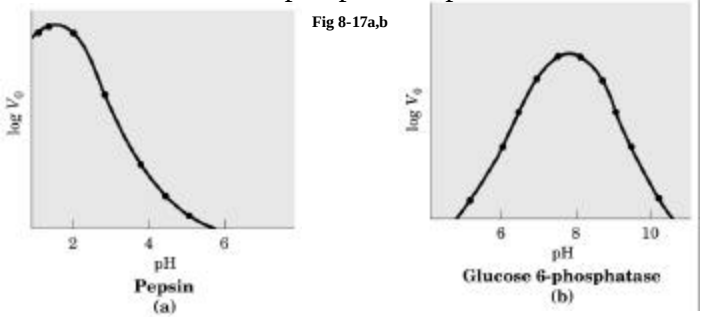
http://www.bio.mtu.edu/campbell/bl482/lectures/lec2/482enz2.htm

Factors that Affect Enzyme Catalysis: pH

(NOT EXTREMES OF pH)

Bell-shaped pH-rate profiles

Fig 8-17a,b



The enzyme active site has a minimum of two functional groups.

For the maximum rate:

One group is required in the conjugate acid form, AH

One group is required in the conjugate base state, B:

rate = k[E-AH][E-B:]

rate = k[E-AH][E-B:]

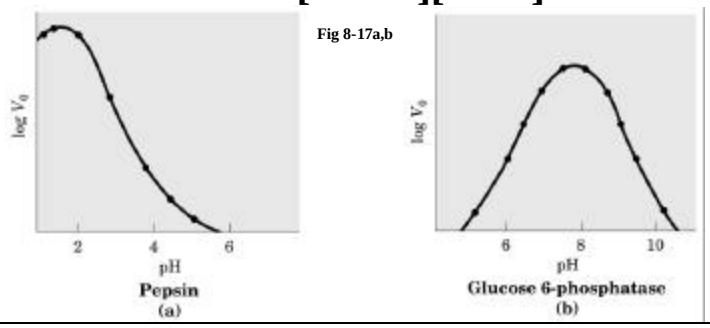


Fig 8-17a,b

Examples of AH = -HISH$^+$, -CO_2H, -SH, -NH_3^+, etc

Examples of B: = -HIS:, -CO_2^-, -S^-, -NH_2, etc

$^+$H:B-**E**-AH	•	:B-**E**-AH	•	:B-**E**-A^-
inactive		active		inactive

Active form has a maximum concentration at the top of the bell-shaped profile

What amino acids are likely to be involved in catalysis?

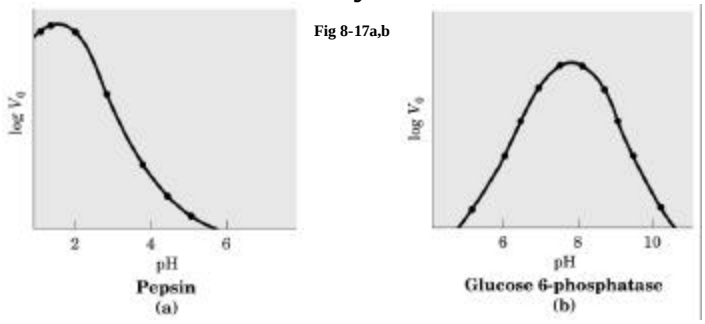
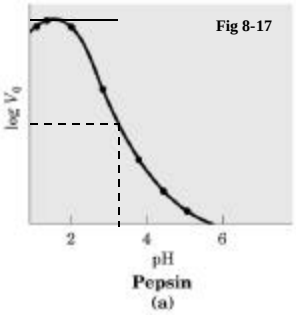


Fig 8-17a,b

$^+$H:B-**E**-AH	•	:B-**E**-AH	•	:B-**E**-A^-
inactive		active		inactive

The pK_a values for the functional groups involved in catalysis can be estimated by looking at the pH values on either side of the bell profile at ½ maximum velocity

PEPSIN?



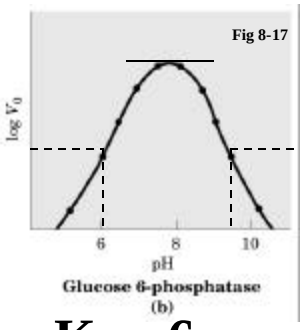
pK_a values indicate very acidic groups

-ASP -- CO_2H

-GLU -- CO_2H

$^+$H:B-E-AH	•	:B-E-AH	•	:B-E-A^-
inactive		active		inactive

GLUCOSE-6-PHOSPHATASE?



pK_a values indicate what amino acids?

pK_a ~ 9.5

LYS or N-terminal (what form?)
CA since rate 9 with pH 8

pK_a ~ 6

HIS (what form?)
CB since rate 8 with pH 8

$^+$H:B-E-AH	•	:B-E-AH	•	:B-E-A^-
inactive		active		inactive

Chymotrypsin Rate % ?

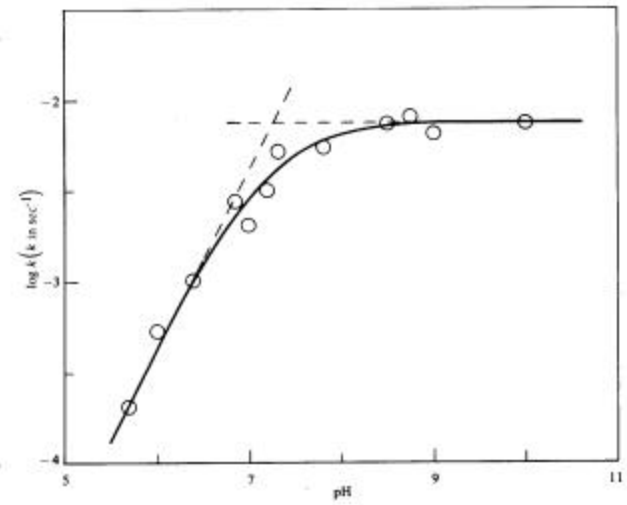


Fig. 5-18. Log of the first-order rate constant of the deacylation of acetylchymotrypsin as a function of pH. (Figure is redrawn from F. C. Wedler, F. L. Killian, and M. L. Bender, *Proc. Nat. Acad. Sci. US* **65**, 1120–1126 (1970).)

Chymotrypsin Rate % E-B:

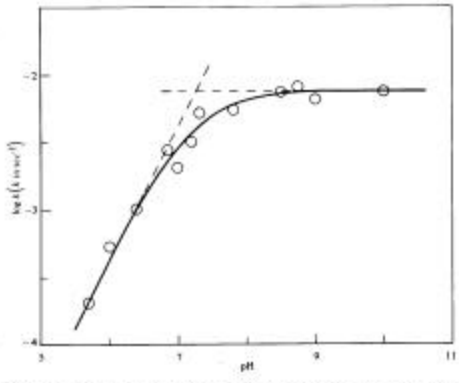
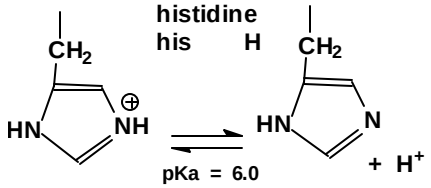


Fig. 5-18. Log of the first-order rate constant of the deacylation of acetylchymotrypsin as a function of pH. (Figure is redrawn from F. C. Wedler, F. L. Killian, and M. L. Bender, *Proc. Nat. Acad. Sci. US* **65**, 1120–1126 (1970).)

What amino acid side chain and what form of this side chain (CA or CB) accounts for this increase in rate as pH is raised?

rate % [E-HIS:]



E-HIS:H⁺ inactive

E-HIS: active

Chymotrypsin Why does rate level off at high pH?

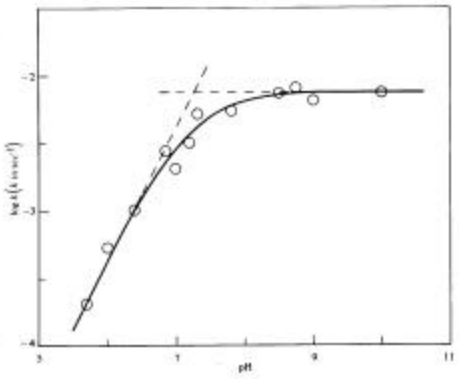
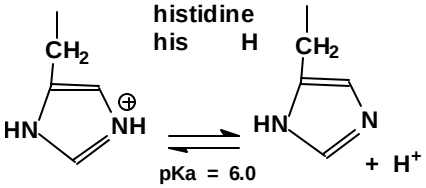


Fig. 5-18. Log of the first-order rate constant of the deacylation of acetyl-chymotrypsin as a function of pH. (Figure is redrawn from F. C. Wedler, F. L. Killian, and M. L. Bender, *Proc. Nat. Acad. Sci. US* **65**, 1120–1126 (1970).)



E-HIS:H$^+$ inactive **E-HIS: active**

rate ∝ [E-HIS:]

[Eo] <<<< [So] at all So; Eo is the limiting reagent.

When all of the E-HIS is in the CB form, the maximum amount of the CB form is present.

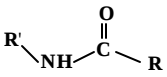
Since the rate [E-HIS], the rate reaches a maximum value.

Problem Set: Notation

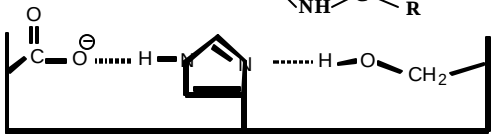
CHEMICAL BASIS OF ENZYME CATALYSIS

In the attached schemes, the enzyme active site is schematically represented with a bold-lined "box". The amino acid side chains that act as functional groups i the site are written in bold lines and letters and are attached to the "box". Substrates are indicated in plain text.

Example substrate:



Example enzyme "box" for chymotrypsin:



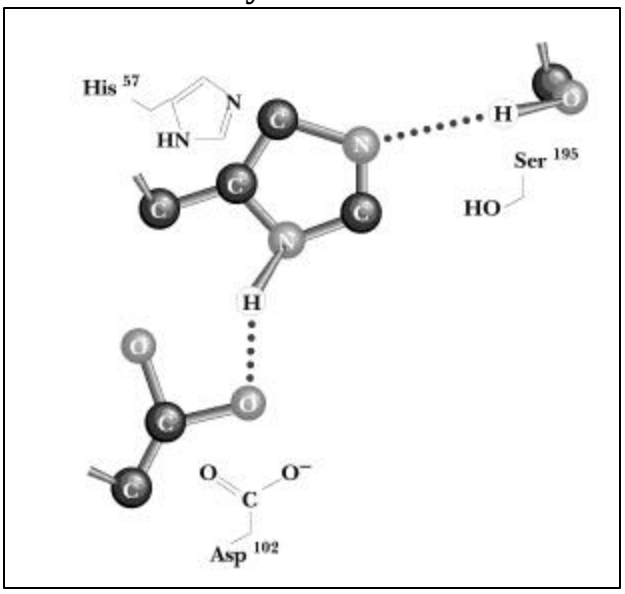
NOTATION: ····· **hydrogen bonding or other weak interactions**

mechanistic arrows

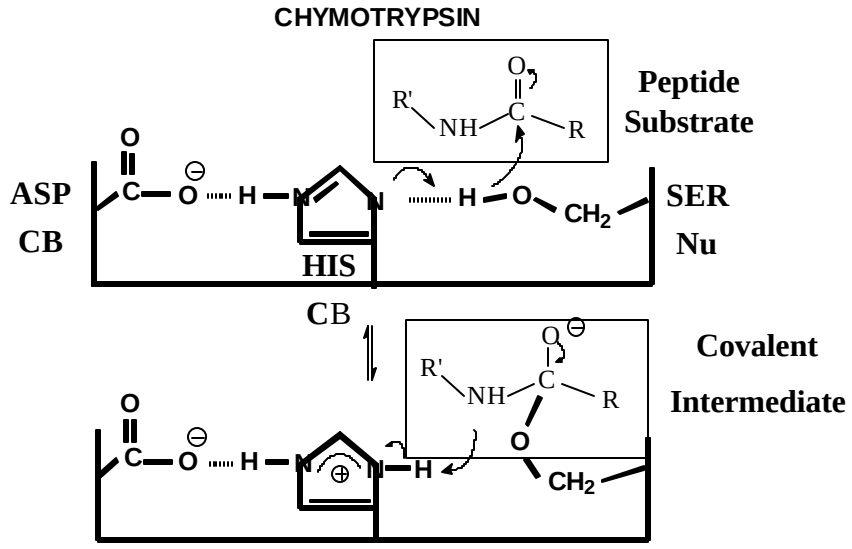
Problem Set: Catalytic Triad

Catalytic Triad of Serine Proteases

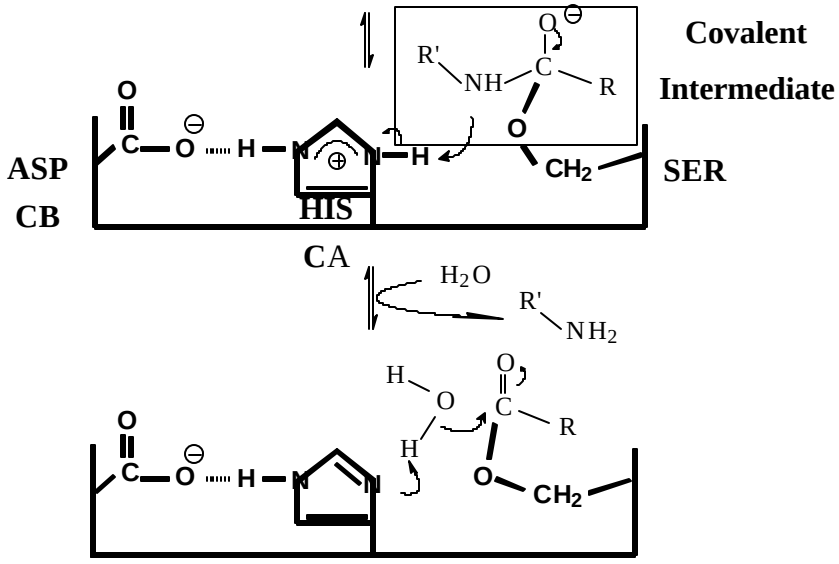
ASP^{-} ··· HIS: ··· SER



Problem Set: Sample Step #1

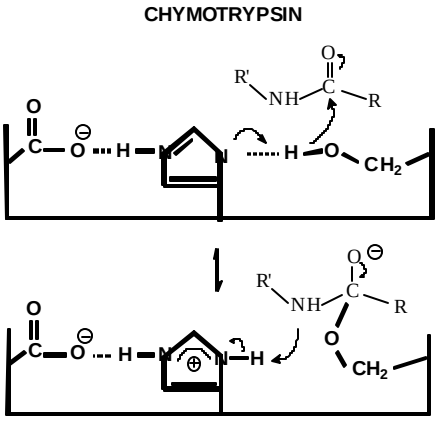


Problem Set: Sample Step #2

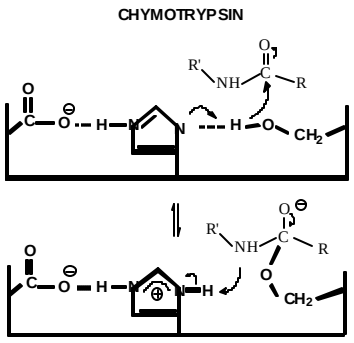


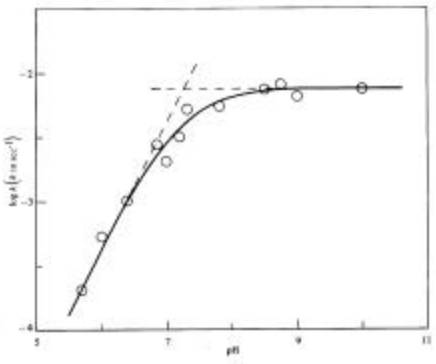
Problem Set: What is function of ASP?

1. **Keeps HIS oriented.**
2. **Raises HIS:H^+ pK_a making CA-HIS:H^+ a poorer acid, but the CB-HIS: a better base to attach the very weak acid HO-SER!**

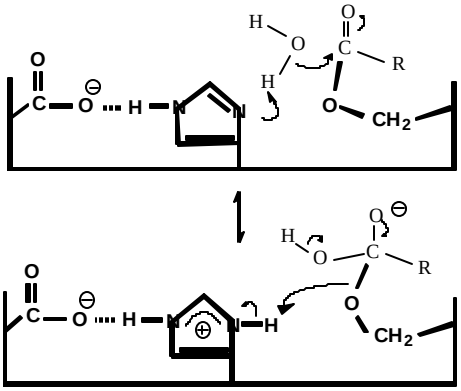


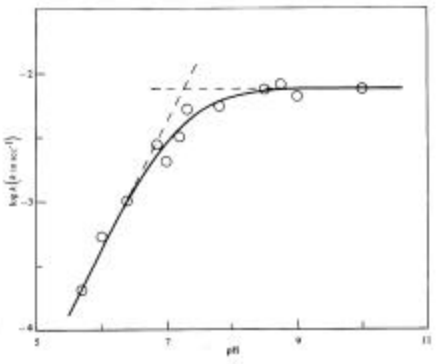
Problem Set: Predict pH-Rate Profile





Deacylation of Chymotrypsin: pH-Rate Profile

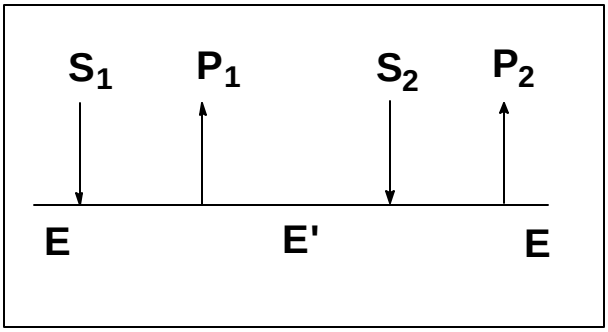




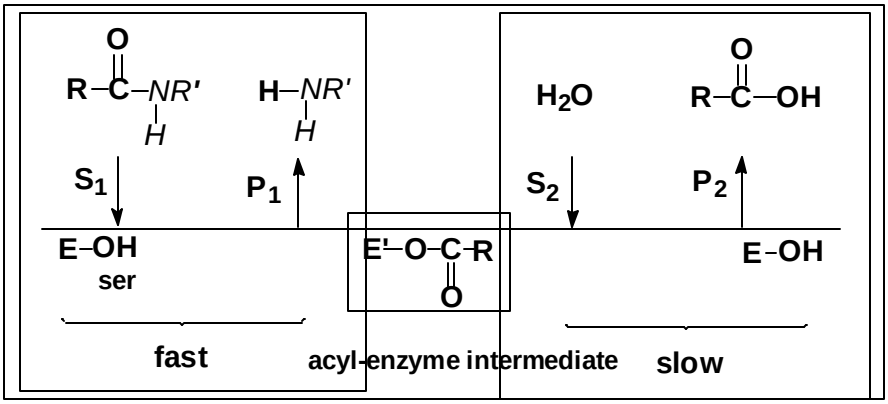
More about Chymotrypsin

CHYMOTRYPSIN CATALYSIS OF PEPTIDE AND ESTER HYDROLYSIS

PING PONG BI BI MECHANISM



Ping Pong Mechanism!

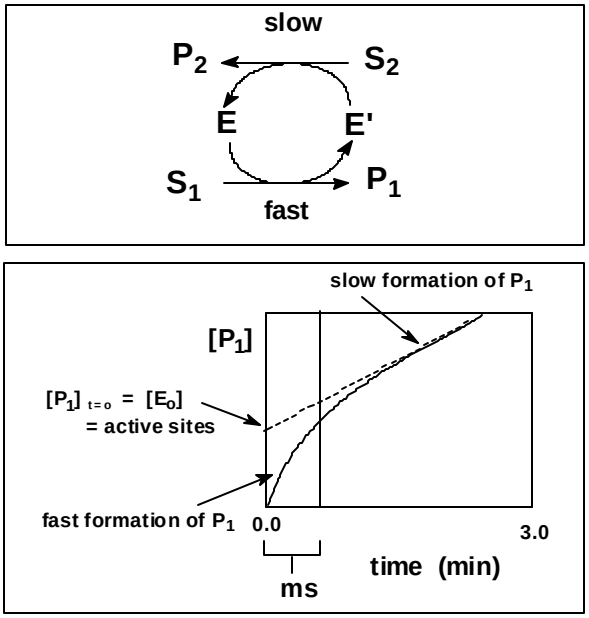


Fast = acylation step

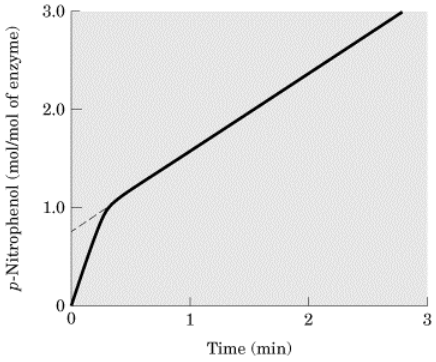
Slow = deacylation step

Acyl enzyme = inactive

Understanding Laboratory Experiment



Read Text about Chymotrypsin



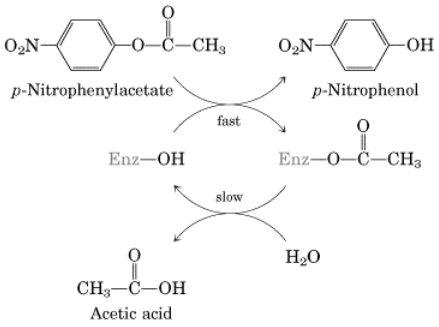
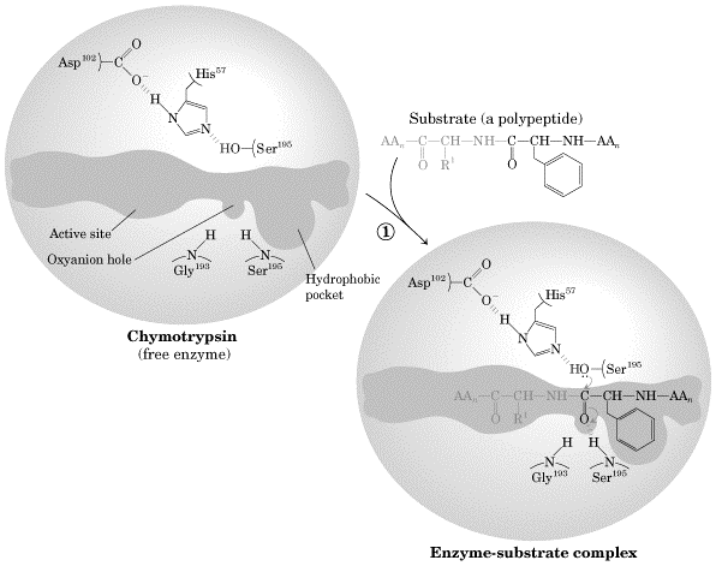


Fig 8-20

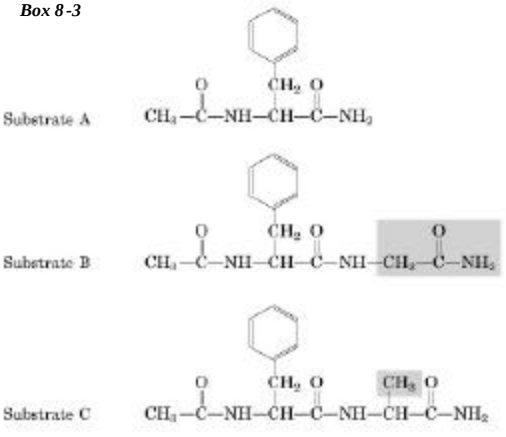
Read Text about Chymotrypsin



etc.

Structure-Activity Relationships

Box 8-3



k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1}s^{-1}$)
0.06	31	2
0.14	15	10
2.8	25	114

B compared to A: B faster, tigher binding

C compared to B: C faster, weaker binding

Enzyme Activity

Vo (rate) at given E_o has units of M min^{-1}

Specific Activity = Rate/mg E_o

$$\frac{M\ min^{-1}}{mg} = \frac{U}{mg}$$

1 U often defined as 1 μmol/min