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**
Conditions that cause protein denaturation:
Urea/Guandinium-HCl

-Addition of urea or guanidinium hydrochloride (G@Cl)
-Urea and G@Cl both H-bond to proteins causing disruption of the H-bond stabilization.
-Proteins unfold and remain soluble (usually).

Addition of soaps or detergents
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 H₂O⁺ 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**

Optimum Temperature

![Graph showing Enzyme Activity vs 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

![Graphs showing Bell-shaped pH-rate profiles](http://www.bio.mtu.edu/campbell/bl482/lectures/lec2/482enz2.htm)

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:

\[
\text{rate} = k[E-AH][E-B]\
\]
rate = $k[E-AH][E-B:]$

Examples of $AH = -\text{HISH}^+, -\text{CO}_2\text{H}, -\text{SH}, -\text{NH}_3^+$, etc

Examples of $B = -\text{HIS}, -\text{CO}_2^-, -\text{S}^-, -\text{NH}_2$, etc

$^+\text{H:B-E-AH} \rightleftharpoons \text{B-E-AH} \rightleftharpoons \text{B-E-A}^-$

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?

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 $\frac{1}{2}$ maximum velocity
**PEPSIN?**

pKₐ values indicate very acidic groups

- ASP -- CO₂H
- GLU -- CO₂H

\[ +H:B-E-AH \quad \rightarrow \quad :B-E-AH \quad \rightarrow \quad :B-E-A^- \]

inactive \quad active \quad inactive

**GLUCOSE-6-PHOSPHATASE?**

pKₐ values indicate what amino acids?

pKₐ ~ 9.5

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

pKₐ ~ 6

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

\[ +H:B-E-AH \quad \rightarrow \quad :B-E-AH \quad \rightarrow \quad :B-E-A^- \]

inactive \quad active \quad inactive
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?

\[
\text{rate} \% [\text{E-HIS:}] \]

E-HIS: H⁺ inactive  \hspace{1cm}  E-HIS: active

\[ pK_a = 6.0 \]
Why does rate level off at high pH?

![Graph showing log of first-order rate constant vs. pH](Fig 5-16)

**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 in the site are written in bold lines and letters and are attached to the "box". Substrates are indicated in plain text.

**Example substrate:**

\[R'\text{NH}-\text{C}^\text{\textcircled{O}}-\text{R}\]

**Example enzyme "box" for chymotrypsin:**

[Box diagram]

**NOTATION:**

- `\cdots`: hydrogen bonding or other weak interactions
- `\rightarrow`: mechanistic arrows

**Problem Set:** Notation

\[\text{[Eo]} \ll \text{[So]} \text{ at all So; Eo is the limiting reagent.}\]

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

Since the rate \([E\text{-HIS}]\), the rate reaches a maximum value.
Problem Set: Catalytic Triad

Catalytic Triad of Serine Proteases

ASP → HIS → SER

Problem Set: Sample Step #1

CHYMOTRYPSIN

Peptide Substrate

Covalent Intermediate

Peptide Substrate
Problem Set: Sample Step #2

**Covalent Intermediate**

1. Keeps HIS oriented.
2. Raises HIS:H⁺ pKₐ 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

$$\begin{align*}
S_1 & \quad P_1 & \quad S_2 & \quad P_2 \\
E & \quad E' & \quad E
\end{align*}$$

Ping Pong Mechanism!

$$\begin{align*}
\text{Fast} = \text{acylation step} & \quad \text{Slow} = \text{deacylation step} \\
\text{Acyl enzyme} = \text{inactive}
\end{align*}$$
Understanding Laboratory Experiment

Read Text about Chymotrypsin

Fig 8-20
Read Text about Chymotrypsin

Structure-Activity Relationships

Box 8-3

<table>
<thead>
<tr>
<th>Substrate</th>
<th>( k_{cat} ) (s(^{-1}))</th>
<th>( k_{m} ) (mM)</th>
<th>( k_{cat}/k_{m} ) (M(^{-1})s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.65</td>
<td>31</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>0.14</td>
<td>36</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>2.8</td>
<td>25</td>
<td>114</td>
</tr>
</tbody>
</table>

* B compared to A: B faster, tighter binding

* C compared to B: C faster, weaker binding
Enzyme Activity

Vo (rate) at given E₀ has units of M min⁻¹

<table>
<thead>
<tr>
<th>Specific Activity = Rate/mg E₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>M min⁻¹ U</td>
</tr>
<tr>
<td>------ = -----</td>
</tr>
<tr>
<td>mg    mg</td>
</tr>
</tbody>
</table>

1 U often defined as 1 μmol/min