Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2018

Lecture 8:

Quiz Questions and Introduction to Proteases

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A Quiz Question from 2017

- The stated question:
  - A buffer prepared by mixing:

    0.2 moles HEPES, a weak acid with a pK_a of 7.5.
    0.1 moles Na-HEPES, the conjugate base of HEPES
    water to a final volume of 600 mL
  - What is the expected pH?
- Some things to consider:
  - What is this question about?
  - What will the answer look like?
  - What will determine the answer?

Clicker Question #1

$$HA \rightleftharpoons A^- + H^+,\ pK_a = 7.5$$

What happens when HA and Na-A are dissolved in water?

(Choose 1)

1. All of the HA dissociates into A^- and H^+.
2. Most of the HA dissociates into A^- and H^+.
3. Some of the HA dissociates into A^- and H^+, and some of the A^- accepts H^+.
4. Most of the A^- accepts H^+.
5. All of the A^- accepts H^+.

- A better answer: All of the molecules of HA and A^- are in very rapid equilibrium, *but* there is only a small net change in the concentrations after they are dissolved in water.

Clicker Question #2

$HA \rightleftharpoons A^- + H^+$, $pK_a = 7.5$

Mix 0.2 moles HA and 0.1 moles Na-A.

What will the pH be?

1. $pH < 6.5$
2. $6.5 \leq pH < 7.5$
3. $pH = 7.5$
4. $7.5 < pH \leq 8.5$
5. $pH > 8.5$

Calculating the pH

- $HA \rightleftharpoons A^- + H^+$, $pK_a = 7.5$

$$pH - pK_a = \log \frac{[A^-]}{[HA]} = \log \frac{\text{moles } A^-}{\text{moles HA}}$$

- The number of moles A^- and HA are both *very* close to the amounts initially added to the solution, 0.1 moles A^- and 0.2 moles HA.

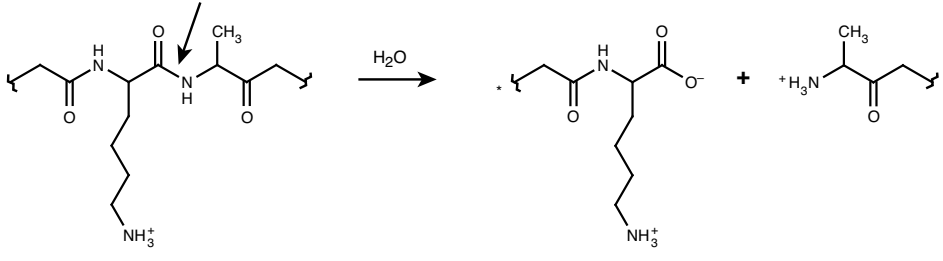
$$\begin{aligned} pH &= pK_a + \log \frac{0.1}{0.2} \\ &= 7.5 - 0.3 \\ &= 7.2 \end{aligned}$$

Warning!

Direction Change

Proteases

The General Protease Reaction

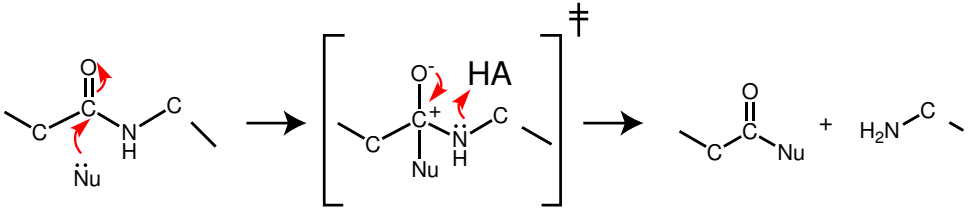


- About 2% of genes in most organisms encode proteases. (Hedstrom, L. 2002, *Chem. Rev.* **102**, 4429)

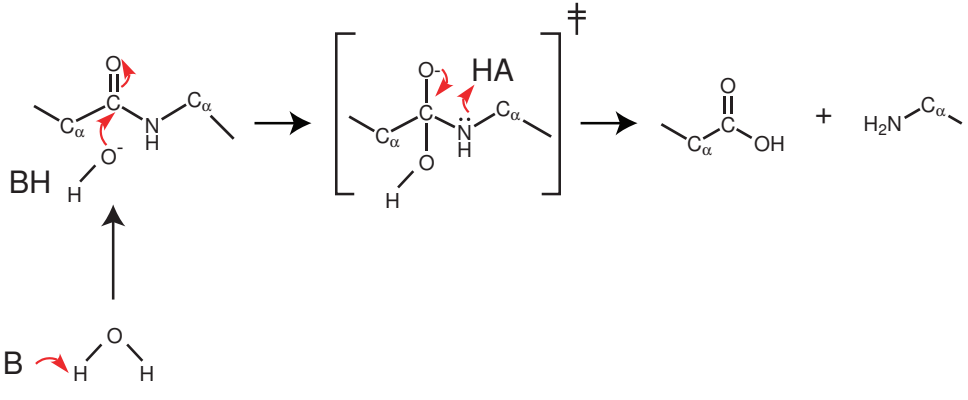
Some Biological Functions of Proteases

- Digestion of food
  - Non-selective
  - Catalyzed by trypsin, chymotrypsin, pepsin and other proteases
- Intracellular protein degradation
  - Highly selective and regulated
  - Often catalyzed by large protein complexes, *e.g.,* the proteasome
- Regulation of biological activity by proteolytic activation
  - Angiotensin converting enzyme (blood pressure regulation)
  - Blood clotting, and disruption of blood clots
  - Complement fixation (an element of the immune response)
  - Apoptosis (programmed cell death)
- Maturation of viral proteins, *e.g.,* HIV and other retroviruses

General Protease Mechanism is Nucleophilic Substitution

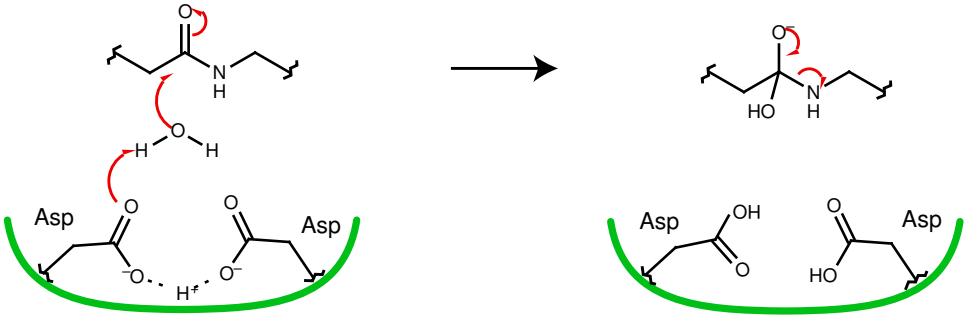


Water Can Act as the Nucleophile, but Must be Activated by a Base



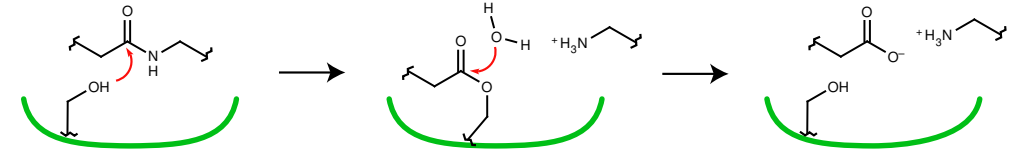
- Why is this reaction so slow in the absence of an enzyme?
- How do enzymes enhance the rate?

Carboxyl Groups Activate H_2O in Aspartyl Proteases



- Examples include pepsin and HIV protease

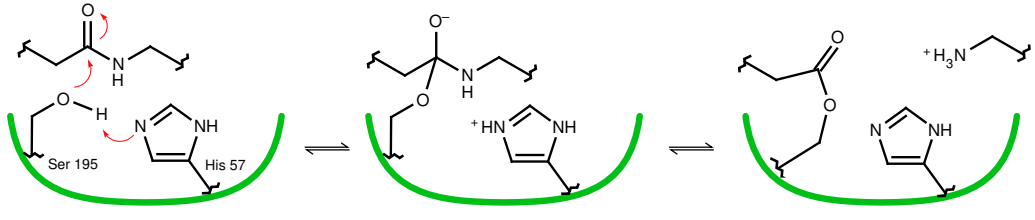
Serine Proteases Employ a Two-Step Mechanism



Enzyme-Substrate Complex | Acyl Intermediate | Enzyme + Products

- In step 1, a serine hydroxyl is the nucleophile.
- In step 2, a water molecule is the nucleophile.
- Both steps require activation by a base.
- Examples include trypsin, chymotrypsin, blood clotting factors and many others.

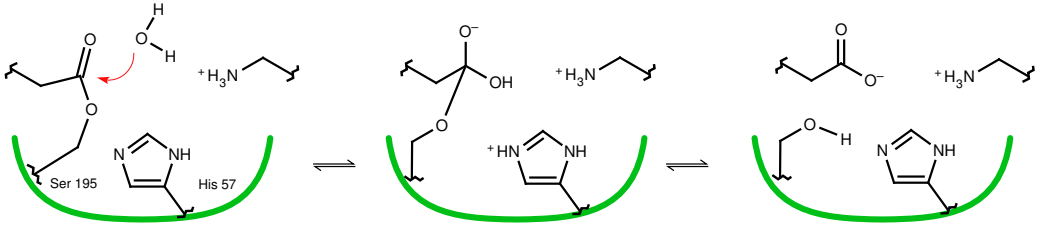
Serine Protease Mechanism: Step 1



Enzyme-Substrate Complex | Tetrahedral Intermediate 1 | Acyl Intermediate

- His 57 is the base that activates the serine hydroxyl.
- The protonated histidine is stabilized by Asp 102 (not shown).
- His 57 acts as an acid to protonate the leaving group.

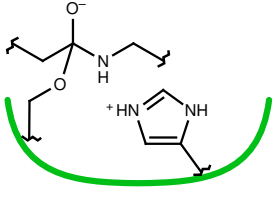
Serine Protease Mechanism: Step 2



- His 57 again acts as a base, to activate the nucleophile (water).
- His 57 again acts as an acid, to re-protonate the Ser hydroxyl.

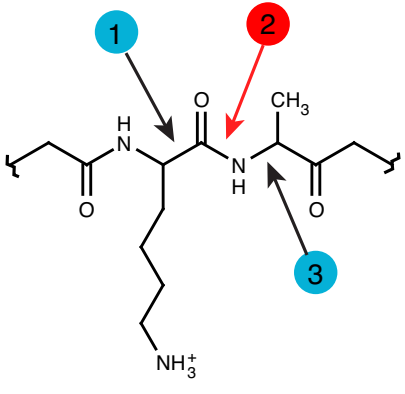
Factors That Contribute to Catalysis

- Activation of nucleophiles by base (His 57).
- Stabilization of protonated His 57 by Asp 102.
- Transfer of proton to leaving group.
- Stabilization of tetrahedral transition state

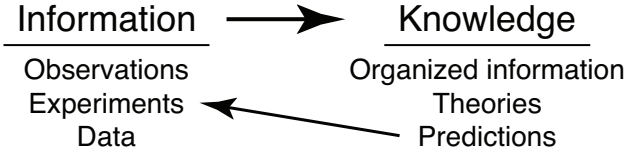


  - Complementary geometry.
  - Electrostatic stabilization of charge on oxyanion.

Clicker Question #3:
What bond does a protease break?



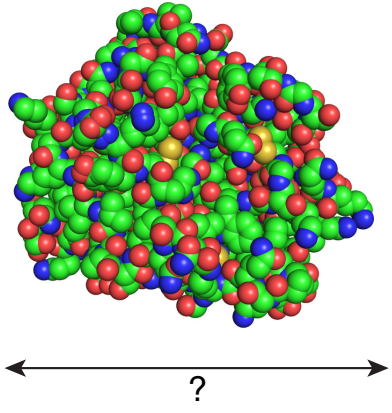
How Do We Know What We Know About Serine Proteases?



- Chemical and biochemical data:
  - Enzyme kinetics
  - Studies with inhibitors
  - Chemical analysis, *e.g.,* active site labeling
- Structural analysis, mostly x-ray crystallography

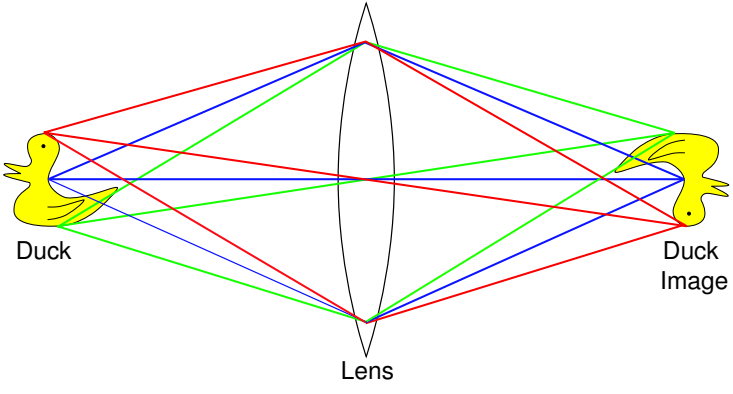
Clicker Question #4: How Big is an Enzyme?

1. 10^{-10} m
2. 10^{-9} m
3. 10^{-8} m
4. 10^{-7} m
5. 10^{-6} m

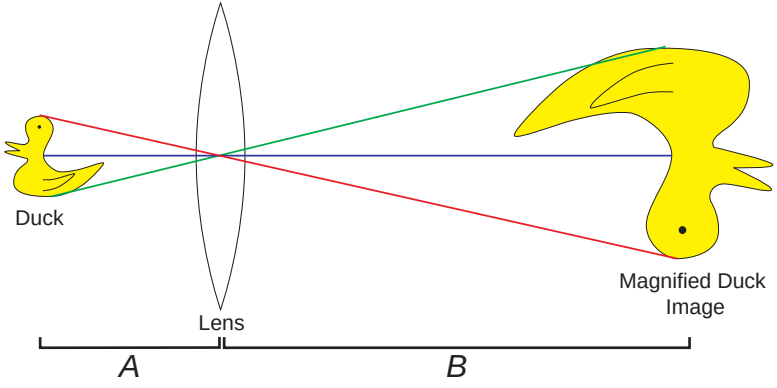


No wrong answers (for now)!

Image Formation with a Lens



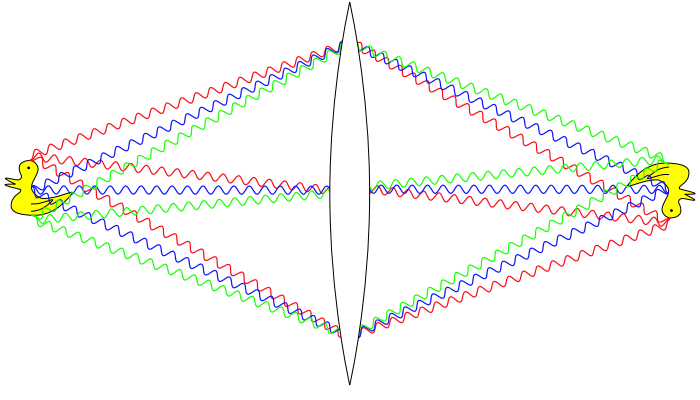
Optical Magnification



As the object is brought closer to the lens

- Image moves further from the lens and becomes larger.
- Magnification $= B/A$
- Magnification, in principle, is not limited, but resolution is.

Imaging With a Lens - a Wave Interpretation



- Image is formed at points where waves are brought back in phase.
- Points in the object must be separated by at least $\sim 1/2$ wavelength to give rise to separate points in the image.