Jennifer Tareila
August 11, 2004
Lesson Plan: Pet Enzyme 2 (Trypsin)

Title: Pet Enzyme kinetics lesson plan- Webquest
Audience: 11th and 12th grade Human Biology (some previous chemistry; all have had biology)
Goals: To discuss the kinetics of trypsin, a digestive enzyme that hydrolyzes proteins
Student Objectives:

At the end of the lesson, students will be able to:

1. Discuss what enzymes kinetics means
2. Illustrate why trypsin cleaves proteins at lysine and arginine only
3. Discuss the types of enzyme regulation

Purpose: To use trypsin as a model protein in regards to structure and regulation

Materials/ Resources:

Computer with Internet access; handout

Prior preparation: verification of web addresses; copies for students of handout questions

Time required: One class period (48 minutes)

Procedure: Student groups are to answer the questions on the handout using the web pages provided, and then discuss questions as a class.

Assessment: Quiz

Web sites: http://www.chembio.uoguelph.ca/educmat/chm356/3560L11.pdf
http://www.dentistry.leeds.ac.uk/biochem/lecture/enzymes/
http://ntri.tamuk.edu/cell/enzyme2.html
http://www.sb.fsu.edu/~hongli/4053NOTES/BCH4053.lec27.ppt
http://departments.oxy.edu/biology/Franck/Bio222/Lectures/Feb22lecture_enzyme_regulation.htm

Name:
Date:

Trypsin and Enzyme Regulation

Use the following sites to help you on your webquest to find out about your pet enzyme:
http://www.chembio.uoguelph.ca/educmat/chm356/3560L11.pdf
http://www.dentistry.leeds.ac.uk/biochem/lecture/enzymes/
http://ntri.tamuk.edu/cell/enzyme2.html
http://www.sb.fsu.edu/~hongli/4053NOTES/BCH4053.lec27.ppt
http://departments.oxy.edu/biology/Franck/Bio222/Lectures/Feb22lecture_enzyme_regulation.htm

1. How does substrate concentration effect reaction rate?

2. Why do enzymes have optimal pH and temperatures? Explain in detail!

3. What controls the specificity of an enzyme?

4. Give the Michaelis-Menton equation for enzyme kinetics. Explain!

5. Compare and contrast competitive and non-competitive enzyme inhibition.

6. How is trypsin regulated? Is this competitive or noncompetitive inhibition?

7. What would happen to the effectiveness of trypsin if the Ser 195 residue were replace with glycine? Explain.

8. Why doesn't trypsin cleave a protein at a histidine?

9. Why does trypsin cleave peptide bonds at Arg and Lys as compared to Tyr? (Hint- look at the structures of each amino acid)

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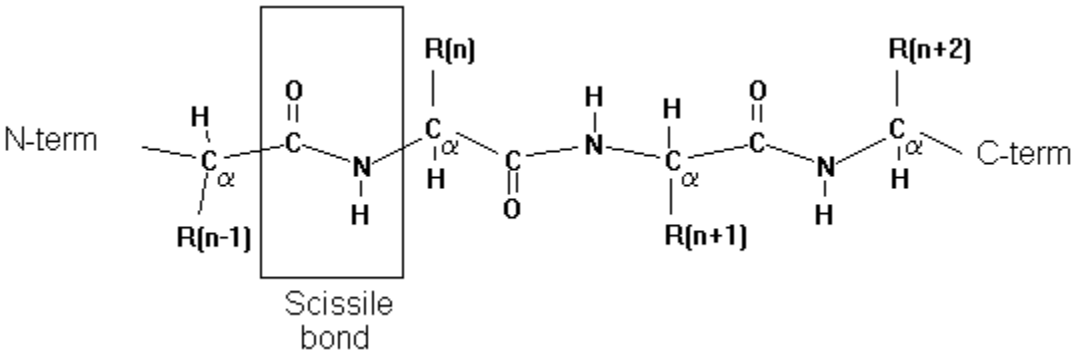
Pet Enzyme Project/ Lesson Plan on Trypsin, part II

(1) Review: What reaction(s) is specifically catalyzed by your enzyme?

Trypsin cleaves proteins at Arg or Lys by cutting at the carboxy terminus at these amino acids; in addition it activates other proteases like trypsinogin, chymotrypsinogin, elastase, and procarboxypeptidase. If there are 30 Arg and Lys residues present, there will be 31 peptide fragments created.

The peptide bond is cleaved at lysine or arginine on the carboxy side of the bond, as the Arg and Lys residues fit nicely into the bonding pocket created by the Asp 189, Gly 216, and Gly 226 amino acids.



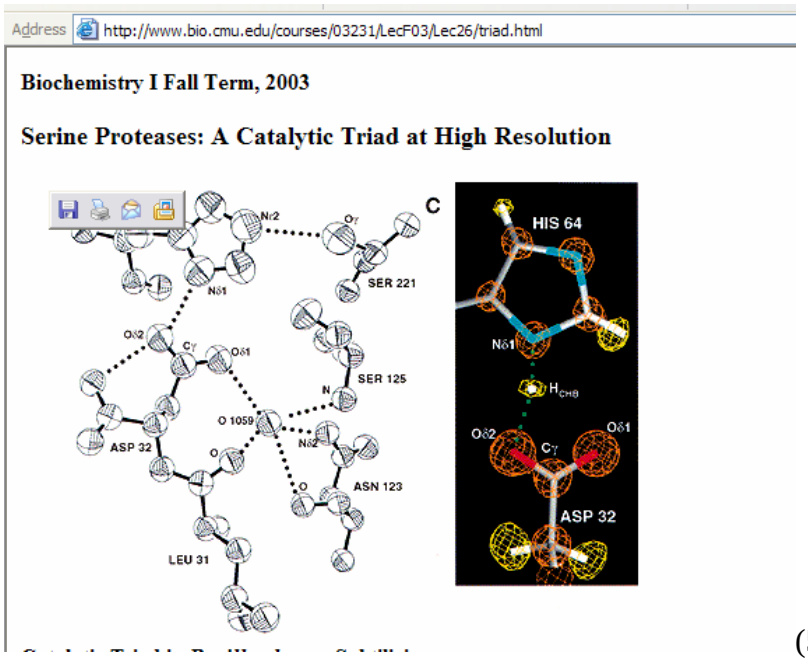
(12)

$H3N^+$- Asp- Ala-Gly-Arg-His-Cys-Lys-Trp-Lys-Ser-Glu-Asn-Leu-Ile-Arg-Thr-Tyr-C

$H3N^+$- Asp-Ala-Gly-Arg-C + $H3N^+$- His-Cys-Lys-C + $H3N^+$- Trp-Lys-C

+ $H3N^+$- Ser-Glu-Asn-Leu-Ile-Arg-C + $H3N^+$- Thr-Tyr-C

(1, 2, 3, 4)

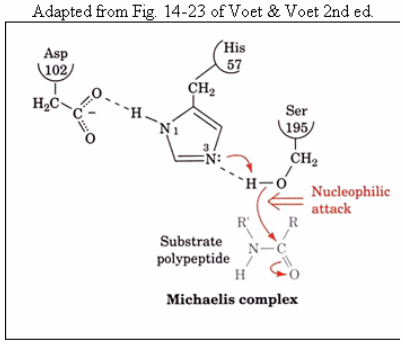


(5)

(2) Give accurate curved-arrow representations of each step.

Mechanism of Serine Proteases

Adapted from Fig. 14-23 of Voet & Voet 2nd ed.



Start Animation Stop Animation

Faster Slower

Present delay (msec): **1 2 0 0**
(Range 100 to 2000 msec)

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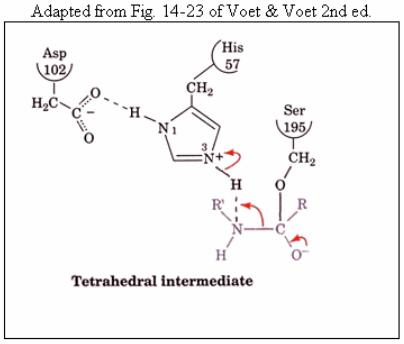

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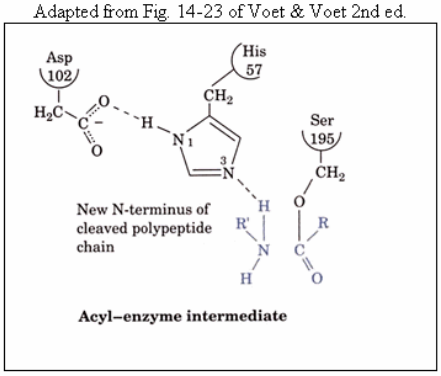

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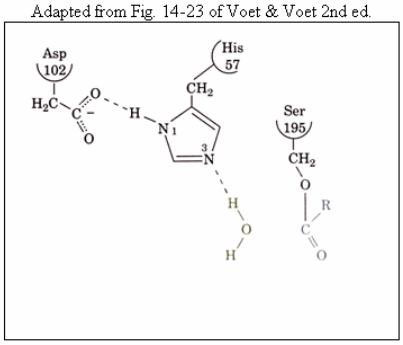

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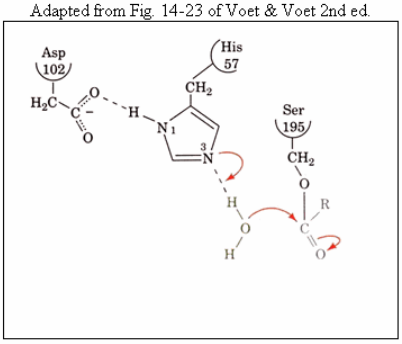

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(Range 100 to 2000 msec)

[Please note: Loading times for the images may vary

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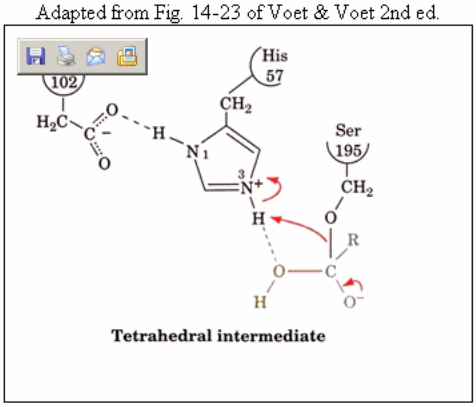

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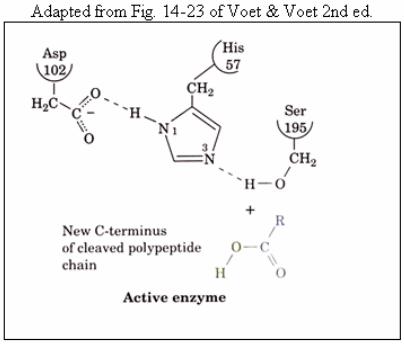

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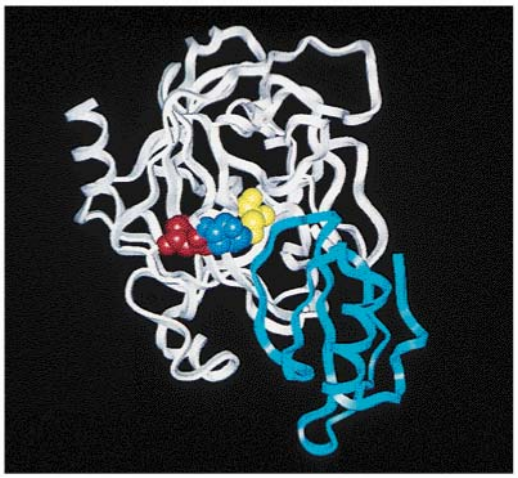
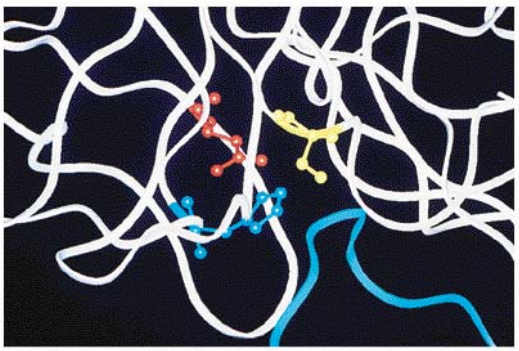
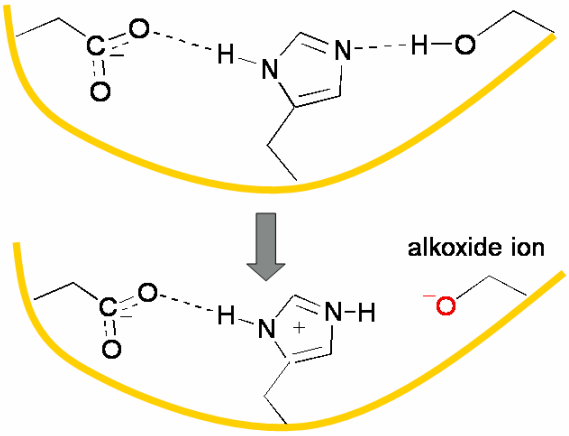
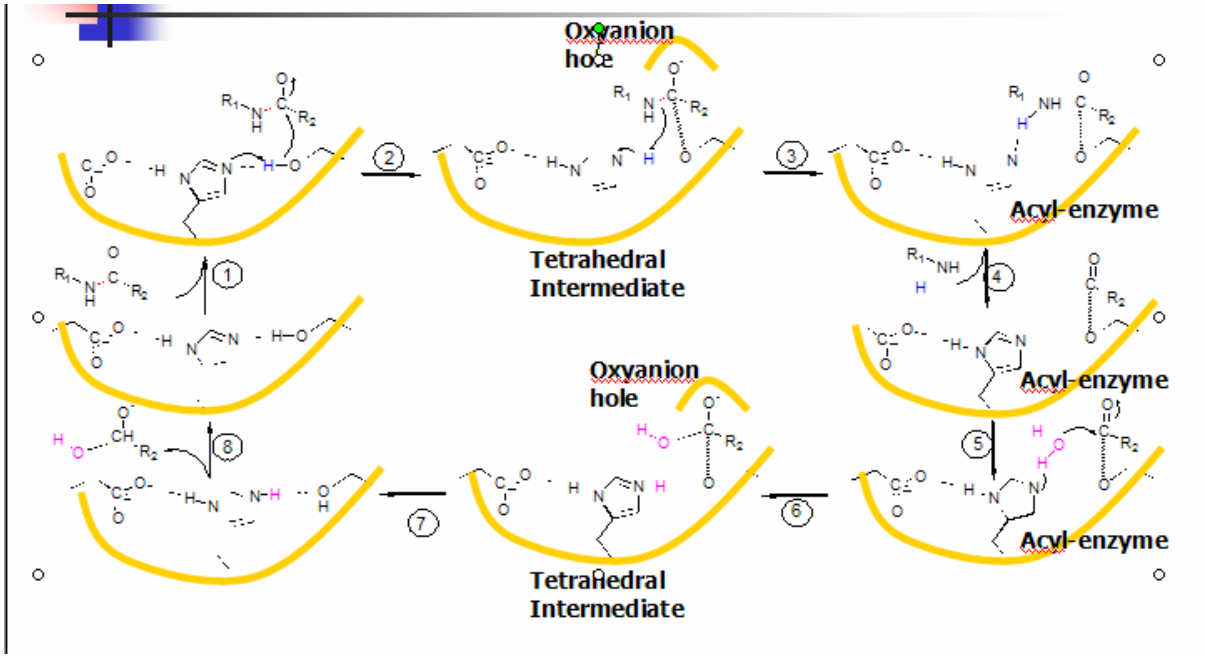




(8)

Asp[102]: Orient His[57] His[57]: General base Ser[195]: Nucleophile





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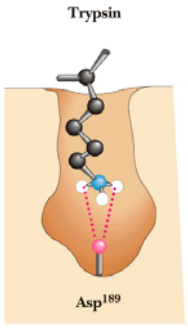

”The binding pocket for the amino acid at the amino end fits an arromatic residue and is surrounded by hydrophobic residues. (comparing with that for trypsin which has an Asp residue to interact with lys). "(8)

(3) What are the catalytic parameters for your enzyme? (needed: it *isn't* necessary to include extensive data under different conditions, etc., unless desirable for your discussion, but representative or typical values of K_M, k_{cat}, and/or more detailed constants for multi-step mechanisms, if these are known, are needed where possible)

The Km for trypsin cleavage of the same bond is 4.7 x 10(-6) M for the arginine isoleucine bond in pig cerebral cortex extracts. (7)

(4) Is your enzyme regulated? If so, how? If it is regulated, why? (What does the regulation accomplish?) If it is not regulated, why not? (Why is regulation apparently not necessary?)

Trypsin is created from activated trypsinogin, and then creates more trypsin. It production is regulated in that fashion; when trypsinogin is produced it does result in the production of trypsin. Also, trypsin can self degrade. In addition, trypsinogin can be deactivated by

changes in pH as the changes in pH change the overall protein structure as the protons (H+) are added/ removed from the trypsin, creating steric hindrance where there once was none, or removing an H+ and allowing bonds to move back. All of these changes result in changing the bonding site of the enzyme, which does regulate the enzyme. Trypsin can be inactivated with trypsin inhibitory factor, which is a competitive inhibitor.(10,11, 12)

RESOURCES

1. Bio220 Exam with posted answers available at http://www.muhlenberg.edu/depts/biology/courses/bio220/keyex1.html Accessed August 8, 2004

2. Chemistry 3560 lectures notes, accessed online at http://www.chembio.uoguelph.ca/educmat/chm356/3560L11.pdf on August 8, 2004.

3. Garrett, Reginald; Grisham, Charles. Principles of Biochemistry with a Human Focus. Brooks/Cole Thomson Learning, USA. 1997. p 96.

4. http://www.med.unibs.it/~marchesi/pps97/course/section12/serprot3.html, accessed 8/15/04

5. http://www.bio.cmu.edu/courses/03231/LecF03/Lec26/triad.html Accessed 8/16/04

6. http://www.bio.cmu.edu/courses/03231/Protease/SerPro.htm, accessed 8/15/04

7. Malesci, A; Straus, E, and Yalow, RS. Proc Natl Acad Sci U S A. 1980 January; 77 (1): 597–599 http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=348321 on August 17, 2004

8. **From** http://www.sb.fsu.edu/~hongli/4053NOTES/BCH4053.lec27.ppt accessed August 17, 2004

9. www.rpi.edu/dept/bcbp/m**o**lbi**o**chem/ MBWeb/mb2/part1/27-pr**o**tease.ppt accessed August 15, 2004

10. Logston, C. J Clin Invest. 2001 November 1; 108 (9): 1267–1268 accessed at http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=209447 on August 16, 2004

11. http://xray.bmc.uu.se/Courses/Bke1/Labs/enz_strfun_lab.html accessed August 16, 2004

12. http://www.whatislife.com/reader/enzyme/enzyme.html accesses August 17, 2004