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Chemistry 504
Lesson Plan 4
Pet Enzyme – Pepsin

Objectives:

Students will be able to:

- A. Describe the shape of the pepsin polypeptide.
- B. Identify the location of the reactive site in pepsin.
- C. Identify the conditions under which pepsin will react.
- D. Identify the principle reaction that pepsin catalyzes and the location in the body where this reaction occurs.
- E. Given the reaction mechanism, identify the proton transfer, intermediate and products of the reaction.
- F. Using experimental results identify the conditions under which the catalyst will perform.
- G. Apply the theory presented to experimental results.

Prior Learning:

Students should have an understanding of protein structure, noncovalent bonding in proteins, peptide bonds and reaction mechanisms. An ability to deduce a mechanism from curved arrow formalism is also necessary.

Teacher Instructions:

Copy “The Pepsin Story” and distribute to the students. Depending on the level of prior learning, the teacher may choose to review the information contained in the hand out before students begin the laboratory experiment. It is anticipated that students will be able to apply the information in “The Pepsin Story” to the analysis questions in their lab. Following the review of “The Pepsin Story”, have students proceed to the experiment, “The pH dependence of Pepsin.”

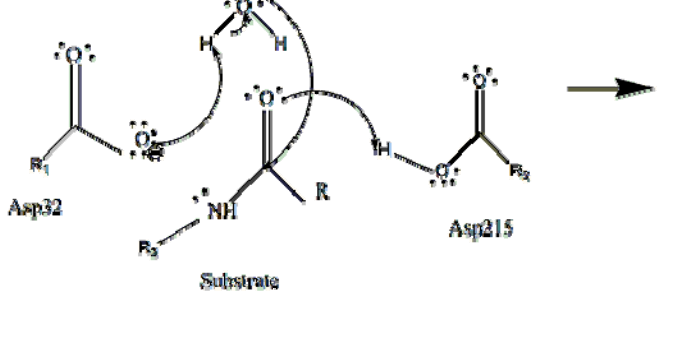
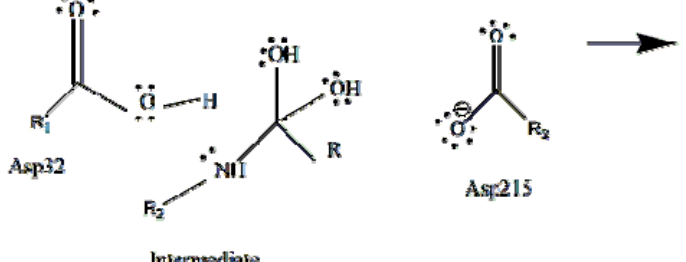
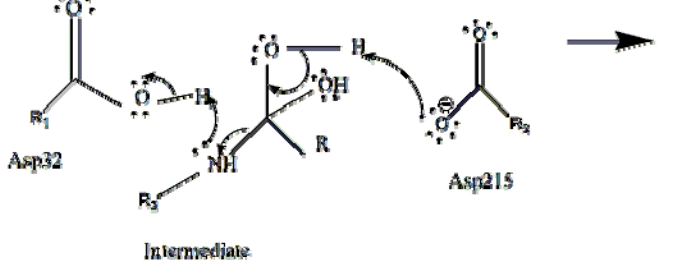
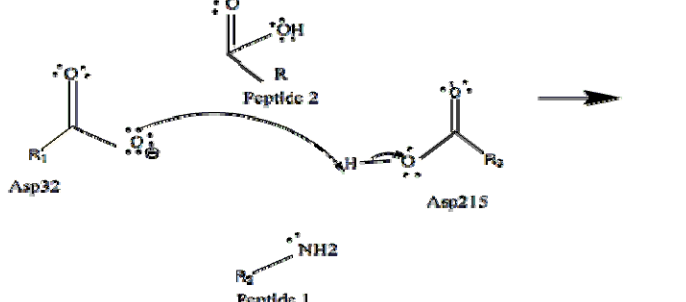
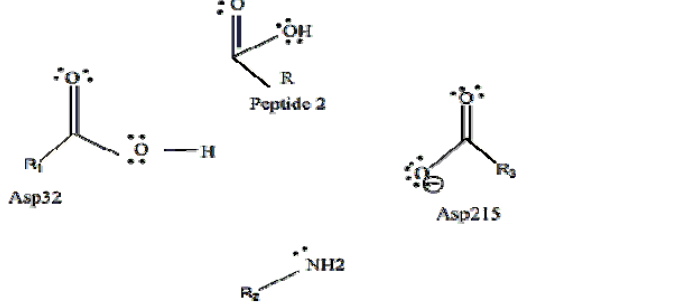
The Pepsin Story

Have you ever given any thought to how all of the food you eat is converted to energy for your body? In order to use the energy from the food, your body must break down the chemicals into small subunits. One of the digestive processes involves the breaking down of larger polypeptides into smaller. The peptide bond is fairly strong and would require a great deal of energy to break under normal conditions. In order to accomplish this task, an enzyme is needed in your stomach that can speed the process along. What is this enzyme? Glad you asked!

Pepsin is an enzyme belonging to the family of aspartic protease enzymes. All members of this class of enzymes have two aspartic acid residues within their structure that act as the active site. For the most part, this class of enzymes is active at acid pH. In the case of pepsin, the pH of optimal activity is extremely acid, between 1 and 4. The specific reaction catalyzed by pepsin is the acid hydrolysis of the peptide bond. This reaction will break down proteins into smaller units to enable the digestive process.

Pepsin demonstrates an unusual property for an enzyme; it does not actually form chemical bonds with its substrate. The unique aspect of the pepsin mechanism is the ability of the two aspartic acids at the reaction site to simultaneously act as both an acid and a base.

The reaction mechanism for the catalyzed hydrolysis of a peptide bond by pepsin:

	<p>Step 1 of the reaction mechanism involves the nucleophilic attack of the carbonyl carbon on the substrate by water. Asp32 accepts a proton from the water. The remaining hydroxyl group from the water attaches to the substrate carbonyl carbon and Asp 215 donates a proton to the substrate</p>
	<p>As a result of step 1, Asp32 is now protonated, and Asp215 is not. The intermediate formed is called an amid dehydrate.</p>
	<p>The next step of the mechanism involves the cleaving of the peptide bond and a proton transfer from ASP32 to the intermediate and from the intermediate to Asp215.</p>
	<p>The proton transfer from Asp 215 to Asp 32 occurs as an equilibrium reaction.</p>
	

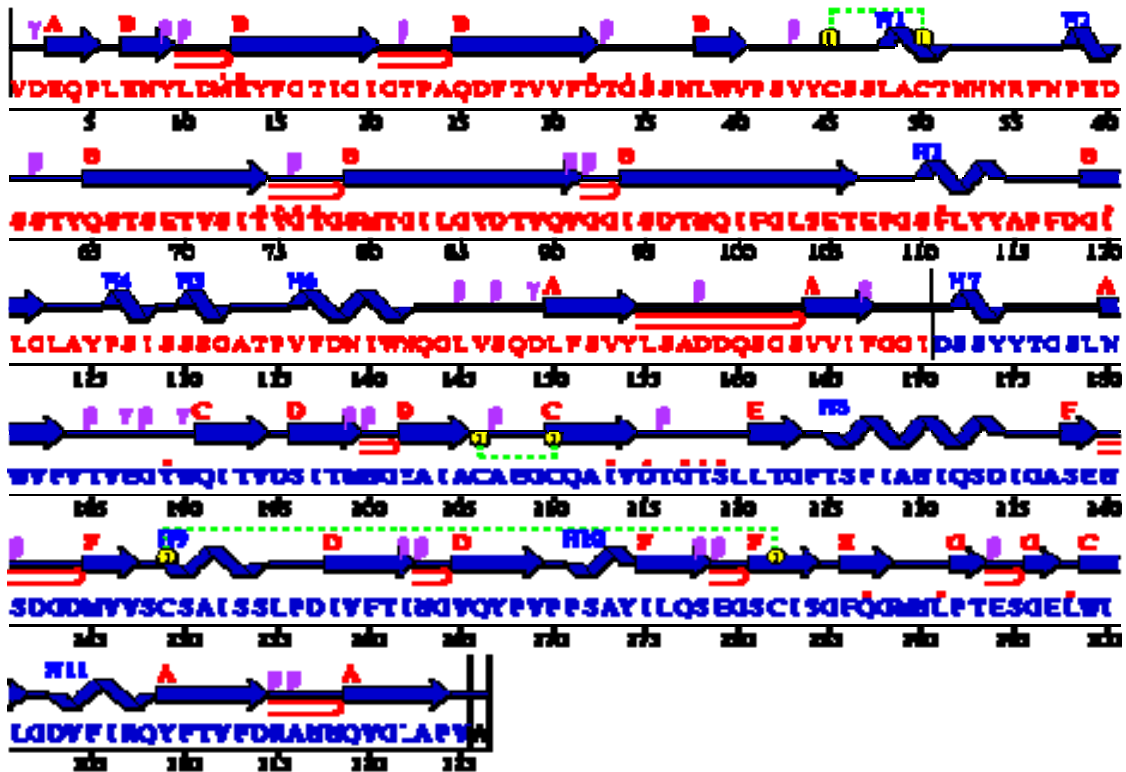
Pepsin is a monomer composed of 326 amino acid residues and has a molecular weight of 34614 Daltons. The primary structure of pepsin is:

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1 VDEQPLENYL DMEYFGTIGI GTPAQDFTVV FDTGSSNLWV PSVYCSSLAC
   EEE EEE TTEEEEEEEEE TTTTEEEEEEE EETT EEE BTT SHHH
51 TNHNRFPED SSTYQSTSET VSITYGTGSM TGILGYDTVQ VGGISDTNQI
   HSS B GGG TTT EE S E EEEE SS EE EEEEEEE EE ETTEEE SEE
101 FGLSETEPGS FLYYAPFDGI LGLAYPSISS SGATPVFDNI WNQGLVSQDL
   EEEESB SH HHHH S SEE EE S GGTG GG HHHHH HHTT SSSE
151 FSVYLSADDQ SGSVVIFGGI DSSYYTGSLN WVPVTVEGYW QITVDSITMN
   EEEE STT S EEEET GGBSS E EEE SSBTTE EEEE EEEET
201 GEAIACAEGC QAIVDGTSL LTGPTSPIAN IQSDIGASEN SDGDMVSCS
   TEESB TT E EEEE TT S EEE HHHHHH HHHHHT EE TTS EE GG
251 AISSLPDIWF TINGVQYPVP PSAYILQSEG SCISGFQGMN LPTESGELWI
   GGGT EEE EETEEEEEE HHHHEEEETT EEEEEEEE B SS B EE
301 LGDVFIRQYF TVFDRANNQV GLAPVA
   E HHHHTTEE EEEETTTTEE EEEEE
  
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<http://www.rcsb.org/pdb/cgi/explore.cgi?job=graphics;pdbId=1QRP;page=;pid=67731122852525&bio=1&opt=show&size=500>

The diagram below shows both the primary and secondary structure for pepsin. The blue arrows represent beta sheets and the curls represent alpha helices.



Residue interaction: - with itself

<http://www.rcsb.org/pdb/cgi/explore.cgi?job=graphics;pdbId=1QRP;page=;pid=67731122852525&bio=1&opt=show&size=500>

Pepsin's secondary structure predominately consists of beta sheets (30). It has a limited number of alpha helicies (6). The ribbon structure shown to the right illustrates this. This model was derived from the PDB file 1QRP, human pepsin. X ray diffraction was resolved at 1.96 Å.

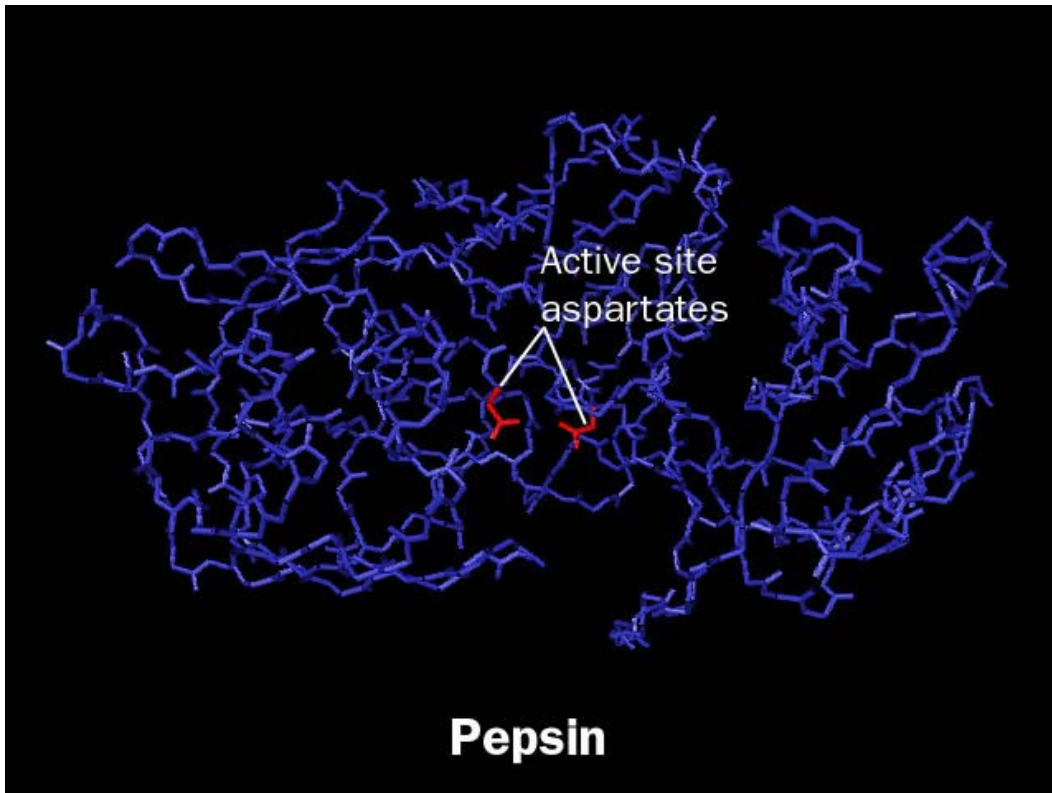


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<http://dwb.unl.edu/Teacher/NSF/C10/C10Links/main.chem.ohiou.edu/~wathen/chem302/protein.html>

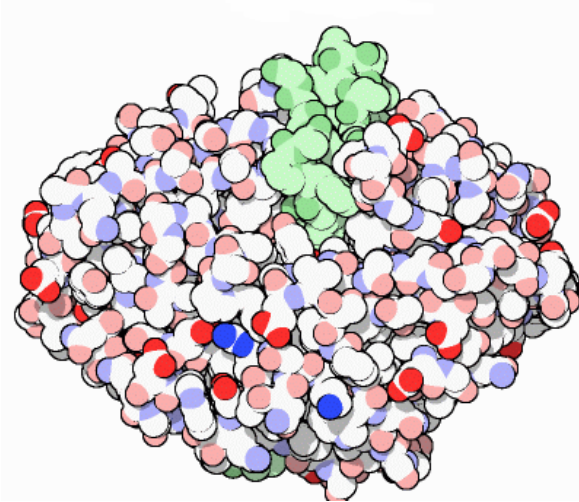
The ribbon structure to the right has been adjusted to show the position of the two aspartic acids composing the reactive site, the first at residue 32 and the second at residue 215.



<http://www.eccentrix.com/members/chempics/Slike/Enzyme/6Pepsin.jpg>

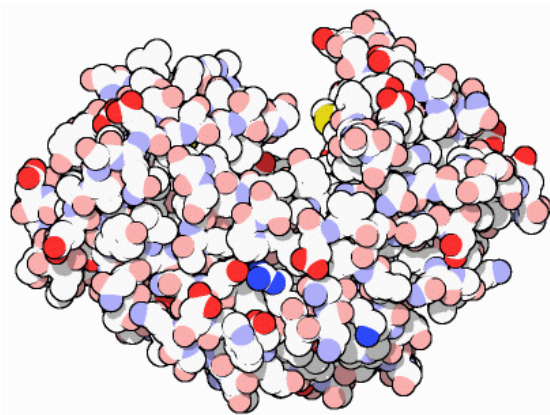
The backbone structure above has been adjusted to illustrate the location of Asp32 and Asp215. As you can see, the general structure consists of one amino acid strand that folds itself into two, almost identical lobes. Enzymes to be cleaved nestle into the active site between the two acid residues.

If pepsin's job is to break down proteins by hydrolyzing the peptide bond, what keeps it from breaking down the proteins of your own body? An answer to this question lies in the fact that before the pepsin is activated, it is formed as pepsinogen, a precursor to pepsin. Pepsinogen is similar in composition to pepsin but it contains 44 additional amino acid residues that prohibit the enzyme reactive site from functioning. The acid conditions of the stomach cause the pepsinogen to alter its structure and become the activated enzyme, pepsin. The top space-filling model below represents pepsinogen. The 44 residue chain is colored green. As you can see, this effectively masks the active site of pepsin, pictured below that. Once the active site is cleared, the enzyme can begin its work on protein cleaving.



Pepsinogen, space-filling, the pepsin precursor

http://www.rcsb.org/pdb/molecules/pdb12_1.html



Pepsin, space-filling

http://www.rcsb.org/pdb/molecules/pdb12_1.html

Enzymes must operate in a specific environment. Since most of your body is composed of water, some parts of the polypeptide must be hydrophilic, or water loving. However, parts of the enzyme must also be hydrophobic. This would enable the enzyme to react with substances that are nonpolar in nature. Kyle-Doolittle Hydropathy Plots graphically display the areas that are hydrophilic and hydrophobic in an enzyme.



The above superimposed graphs indicate both the hydrophobic and hydrophilic areas of pepsin. Although there is significant, regular variation in these properties, as one might expect in a globular protein, a few points can be made. There seems to be a significant hydrophobic area in the middle residues of pepsin. This may account for the fact that pepsin preferentially cleaves proteins with the Phe residue, which is a nonpolar amino acid.

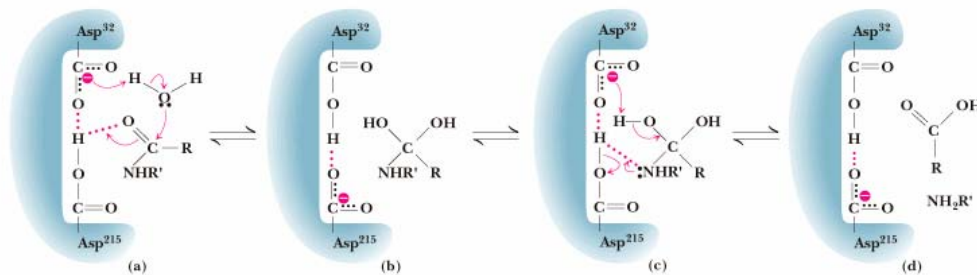
There can be four ligands associated with pepsin.

Identifier Name	Formula
PLE LEUCINE PHOSPHINIC ACID	$C_5 H_{14} N O_2 P$
IVA ISOVALERIC ACID	$C_5 H_{10} O_2$
HFA ALPHA-HYDROXY-BETA-PHENYL-PROPIONIC ACID	$C_9 H_{10} O_3$
CH3 METHYL GROUP	$C H_3$

<http://pd-beta.rcsb.org/pdb/explore.do?structureId=1qrp>

The reaction mechanism for pepsin is pictured below:

Garrett & Grisham: Biochemistry, 2/e
Figure 16.27



Saunders College Publishing

In figure (a) above, we see the carboxyl ends of the aspartic acid residues at 215 and 32. Even though they have the same formula, they have different pK_a values. This is due to the different residues surrounding each aspartic acid. In figure a, the Asp32 is deprotonated while Asp215 still retains its proton. Water will nucleophilically attack the carbonyl carbon of the substrate while Asp32 accepts a proton from the water and Asp215 donates a proton to the carbonyl carbon of the substrate. The intermediate that is formed is called an amide hydrate. This intermediate decomposes by Asp32 now accepts a proton from the intermediate as Asp215 donates its proton to the amid. This action cleaves the peptide bond in the substrate. The final step of the mechanism, not pictured in the diagram above is one in which the proton on Asp32 is transferred to Asp215.

Pepsin is a fairly well characterized enzyme. In 1930, John Northrup crystallized it and from his work with pepsin was able to prove that enzymes were proteins. As a result of this well known structure, many catalytic studies have been conducted on this enzyme. In 1975, Sachdev and Fruton studied the kinetics of pepsin on fluorescent substrates. The table following lists a sample of some kinetic values for pepsin. As a result of this study, the authors conclude that kinetic values of pepsin are dependent upon the identity of the substrate; not just the immediate identity of the residues on either side of the peptide bond to be cleaved, but also residues further down the substrate chain.

Table 1. Kinetics of cleavage of mansyl and dansyl peptides by pepsin

Substrate	Steady-state kinetics				Stopped-flow kinetics			
	[E] _t (μM)	[S] ₀ (μM)	k _{cat} (sec ⁻¹)	K _m (μM)	[E] _t (μM)	[S] ₀ (μM)	k ₂ (sec ⁻¹)	K _s (μM)
Mns-Phe-Phe-OP4P	10	30-90	0.002	95	—	—	—	—
Mns-Gly-Phe-Phe-OP4P	0.2	14-109	0.13	37	5-30	1.0	0.10	34
Mns-Gly-Gly-Phe-Phe-OP4P	0.003	15-113	16	75	25-75	5.0	13	94
Dns-Gly-Gly-Phe-Phe-OP4P	0.01	14-134	4.4	109	10-50	5.0	4.6	97
Dns-Gly-Ala-Phe-Phe-OP4P	0.0012	23-218	34	60	10-50	5.0	146	57
Dns-Ala-Ala-Phe-Phe-OP4P	0.0004	18-111	91	54	10-50	5.0	430	57
Mns-Ala-Ala-Phe-Phe-OP4P	0.0004	21-110	112	65	—	—	—	—

Now that you are familiar with the background theory about the enzyme pepsin, perhaps it would be worthwhile to determine some experimental parameters. Follow the lab instructions given by your teacher.

References

Garrett and Grisham, Principles of Biochemistry with a Human Focus, Brooks/Cole, 1997

Garrett and Grisham, Biochemistry, Saunders, 2nd edition

Sachdev, G. and Fruton, J., Kinetics of Action of Pepsin on Fluorescent Peptide Substrates, Proc. Of the National Academy of Science, USA, Vol. 72, No.9, pp. 3424-3427, September 1975.

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<http://www.eccentric.com/members/chempics/Slike/Enzyme/6Pepsin.jpg>

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<http://www.rcsb.org/pdb/cgi/explore.cgi?job=graphics;pdbId=1QRP;page=;pid=67731122852525&bio=1&opt=show&size=500>