7.016 Recitation 3 – Fall 2018

*(**Note:** The recitation summary should NOT be regarded as the substitute for lectures)*

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Summary of Lecture 4 (9/12):

Enzymes and energy: Enzymes are biological catalysts. Most biological catalysts are proteins, although RNA can also have enzymatic role and catalytically- active RNAs are known as ribozymes. Enzymes catalyze specific biological reactions and act by lowering the activation energy of the reaction that they catalyze. Each enzyme has a specific 3-D conformation and an active site(s) to which the substrate molecules can bind to form a transition state enzyme-substrate complex (ES complex). The complex then gives rise to product (P) and the enzyme is released in its original form to catalyze the reaction once again.

There may be different forms of the same enzymes (**isozymes**) that have different physical properties but which catalyze the same reaction.

Each enzyme is specific for a particular reaction. Enzyme function may be regulated by various factors. These factors may include i.e. prosthetic groups, metal ions/cofactors, coenzyme, substrate concentration, pH, temperature, inhibitors, competitive or non-competitive inhibitors, allosteric modulators and the feedback inhibition by the end product of a biochemical reaction that involves multiple reaction steps.

Reaction kinetics: An endergonic reaction (one with a positive ΔG) cannot occur spontaneously, although it may be coupled to an exergonic reaction. In some cases exergonic reaction (one with a negative ΔG) can proceed spontaneously. The thermodynamics of the reaction are dictated by the difference in free energy between the substrate and the products. The kinetics of the reaction is determined by the transition stage and how much energy must be added to form the high energy intermediate. Enzymes lower the activation energy needed for a reaction to proceed, but do not change the free energy (ΔG) of either the reactants or the products. The following equation relates ΔG to enthalpy (ΔH, the available energy) and entropy (ΔS, unusable energy).

$$\Delta G = \Delta H - T\Delta S \text{ where T is the absolute temperature.}$$

Questions:

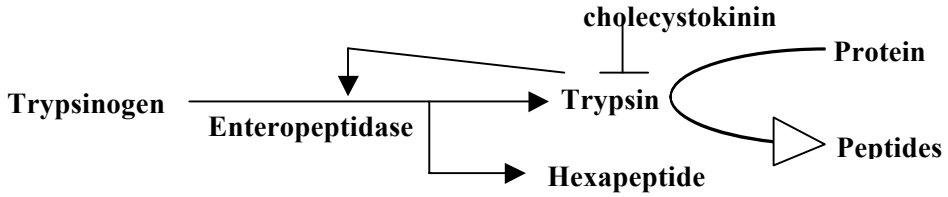
1. Leu-Enkephalin is a bioactive pentapeptide (5 amino acids) that is involved in natural pain regulation. **The sequence is: Tyr-Gly-Gly-Phe-Met**

a) Draw an accurate chemical structure of Leu-enkephalin using a “line-angle” rendition.

b) Label the following on your structure: the C-terminus, the N-terminus, the aromatic side chains of the residues, the a-carbons and the peptide bonds.

2. Trypsin is a protease. This enzyme breaks a protein into peptides by hydrolyzing the peptide bonds that have amino acids lysine and arginine at the carboxyl (-COOH) side of the peptide bond. The steps involved in the production and regulation of trypsin are outlined and also shown in the schematic below. *Please note that a "T" represents inhibition and an "->" represents activation.*

- **Reaction 1:** Trypsin is produced as inactive trypsinogen.
- **Reaction 2:** Trypsinogen is cleaved to active trypsin and a hexapeptide by the action of enteropeptidase.
- **Reaction 3:** Trypsin hydrolyzes the peptide bonds that have lysine and arginine at the carboxyl (-COOH) side of the cleaved peptide bond.
- **Reaction 4:** Trypsin then undergoes feedback inhibition by cholecystokinin.



a) Summarize the effect of enteropeptidase on the reaction parameters specified in the table below.

Parameters	**Enteropeptidase**
Is the reaction catabolic/ anabolic?	
What is the effect of enzyme on the reaction equilibrium (K_{eq}) *(increases/ decreases/ unchanged)?*	
What is the effect of enzyme on the enthalpy of reaction (H) *(increases/ decreases/ unchanged)?*	
What is the effect of enzyme on the entropy of reaction (S) *(increases/ decreases/ unchanged)?*	

You mimic Reaction #3 in five separate test tubes (1-5) as described below. You allow the reaction to proceed for 30 minutes in each tube and measure the amount of protein hydrolyzed.

- ***Tube #1 (Control):*** *You perform the reaction at **37^{o}C** and **pH 7.4** and measure 100% hydrolysis of the protein into peptides.*

- ***Tube #2:*** *You perform the reaction at **50^{o}C** and **pH 7.4** and observe 0% hydrolysis of the protein substrate. However, if the temperature is brought to **37^{o}C,** you observe 100% hydrolysis of the protein as seen in tube #1.*

- ***Tube #3:*** *You perform the reaction at **37^{o}C** and **pH 7.4** for and in the presence of EGTA, a Ca^{++} ion chelator (i.e. it quenches / removes the Ca2+ ions) and observe 0% hydrolysis of the protein substrate. You add excess of Ca^{++} ions to the tube and observe 100% hydrolysis of the protein.*

- ***Tube #4:*** *You perform the reaction at **37^{o}C** and a **pH of 7.4** in the **presence of soybean trypsin inhibitor (SBI)** and do not detect any measurable hydrolysis of the protein. You increase the substrate concentration by 4 fold and observe 100% hydrolysis of the protein.*

- ***Tube #5:*** *You perform the reaction at **37^{o}C** and a **pH of 7.4** in the **presence of di-isopropylfluorophosphate (DFP)** and observe 0% hydrolysis of the protein. You increase the substrate concentration by 4 fold but do not observe a measurable hydrolysis of protein substrate.*

b) Explain the effect of the changed reaction parameters in the following test tubes on **structure** and **function** of trypsin and its protein substrate.

Reaction parameters	Affects **Trypsin (***Yes/No*)? **Explain.**	Affects **Trypsin substrate** (*Yes/ No*)? **Explain.**
50^{o}C in tube #2		
EGTA (a chemical that quenches or removes the calcium) in tube #3		

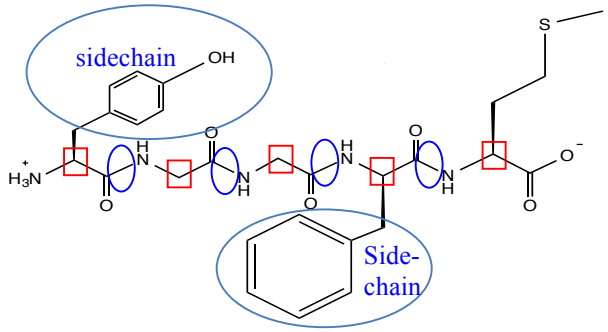
c) Based on the information provided, would you characterize…

i. SBI as a *competitive / non- competitive inhibitor / allosteric modulator*? **Explain** why you selected this option.

ii. DFP as a *competitive/ non- competitive inhibitor / allosteric modulator?* Choose **all possible options** and give an explanation for the option(s) that you selected.

Solutions to Questions:

1. Leu-Enkephalin is a bioactive pentapeptide (5 amino acids) that is involved in natural pain regulation. **The sequence is: Tyr-Gly-Gly-Phe-Met**



a) Draw an accurate chemical structure of Leu-enkephalin using a "line-angle" rendition.

b) Label the following on your structure: the C-terminus, the N-terminus, the aromatic side chains of the residues, the a-carbons and the peptide bonds.

2. Trypsin is a protease. This enzyme breaks a protein into peptides by hydrolyzing the peptide bonds that have amino acids lysine and arginine at the carboxyl (-COOH) side of the peptide bond. The steps involved in the production and regulation of trypsin are outlined and also shown in the schematic below. *Please note that a "T" represents inhibition and an "->" represents activation.*

- **Reaction 1:** Trypsin is produced as inactive trypsinogen.
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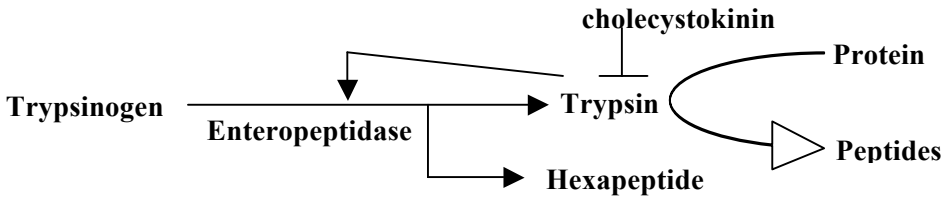
- **Reaction 4:** Trypsin then undergoes feedback inhibition by cholecystokinin.



a) Summarize the effect of enteropeptidase on the reaction parameters specified in the table below.

Parameters	**Enteropeptidase**
Is the reaction catabolic/ anabolic?	***Catabolic***
What is the effect of enzyme on the reaction equilibrium (K_{eq}) *(increases/ decreases/ unchanged)?*	***Unchanged***
What is the effect of enzyme on the enthalpy of reaction (H) *(increases/ decreases/ unchanged)?*	***Unchanged***
What is the effect of enzyme on the entropy of reaction (S) *(increases/ decreases/ unchanged)?*	***Unchanged***

You mimic Reaction #3 in five separate test tubes (1-5) as described below. You allow the reaction to proceed for 30 minutes in each tube and measure the amount of protein hydrolyzed.

- ***Tube #1 (Control):*** *You perform the reaction at* ***37°C*** *and* ***pH 7.4*** *and measure 100% hydrolysis of the protein into peptides.*
- ***Tube #2:*** *You perform the reaction at* ***50°C*** *and* ***pH 7.4*** *and observe 0% hydrolysis of the protein substrate. However, if the temperature is brought to* ***37°C,*** *you observe 100% hydrolysis of the protein as seen in tube #1.*
- ***Tube #3:*** *You perform the reaction at* ***37°C*** *and* ***pH 7.4*** *for and in the presence of EGTA, a* Ca^{++} *ion chelator (i.e. it quenches / removes the Ca2+ ions) and observe 0% hydrolysis of the protein substrate. You add excess of* Ca^{++} *ions to the tube and observe 100% hydrolysis of the protein.*
- ***Tube #4:*** *You perform the reaction at* ***37°C*** *and a* ***pH of 7.4*** *in the* ***presence of soybean trypsin inhibitor (SBI)*** *and do not detect any measurable hydrolysis of the protein. You increase the substrate concentration by 4 fold and observe 100% hydrolysis of the protein.*
- ***Tube #5:*** *You perform the reaction at* ***37°C*** *and a* ***pH of 7.4*** *in the* ***presence of di-isopropylfluorophosphate (DFP)*** *and observe 0% hydrolysis of the protein. You increase the substrate concentration by 4 fold but do not observe a measurable hydrolysis of protein substrate.*

b) Explain the effect of the changed reaction parameters in the following test tubes on **structure** and **function** of trypsin and its protein substrate.

Reaction parameters	Affects **Trypsin (***Yes/No***)? Explain.**	Affects **Trypsin substrate** (*Yes/ No*)? **Explain.**
50°C in tube #2	*Yes, it may denature the enzyme i.e. disrupt its active 3D- dimensional conformation.*	*Yes, since the substrate is a protein itself, an alteration in temperature may disrupt its 3D folding.*
EGTA (a chemical that quenches or removes the calcium) in tube #3	*Ca^{2+} ions may act as a prosthetic group for trypsin. Their chelation by EGTA may prevent trypsin from catalyzing this reaction.*	*No. However, if you make the assumption that the Ca^{2+} ions are needed for the proper folding of protein substrate then your answer is "yes".*

c) Based on the information provided, would you characterize…

i. SBI as a *competitive / non- competitive inhibitor / allosteric modulator*? **Explain** why you selected this option.

The effect of the inhibitor may be reversed by increasing the substrate concentration i.e. it competes with the substrate to bind to the active site of the enzyme. So SBI is a competitive inhibitor.

ii. DFP as a *competitive/ non- competitive inhibitor / allosteric modulator?* Choose **all possible options** and give an explanation for the option(s) that you selected.

The effect of DFP cannot be reversed by increasing the substrate concentration, suggesting that it binds to a site on an enzyme other than the substrate-binding site. This makes DFP either a noncompetitive inhibitor or an allosteric inhibitor.

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7.016 Introductory Biology
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