CH 102 Laboratory 8
Salivary Amylase: An enzyme on the tip of your tongue

INTRODUCTION

When cells need to undertake a chemical reaction they cannot use the same tools as the organic chemist. They cannot use the extremely rare or reactive chemicals that are commonplace in organic synthesis and the range of temperatures and pH's available have to be within a more narrow range than what can be made to occur in a glass reaction flask. The process of biological evolution has come up with a very different, and successful, approach to making chemical change; the needed chemical reactions are carried out using protein catalysts, called enzymes.

Enzymes are all made up with the same 20 building blocks called amino acids, which have the general structure shown below:

\[
\text{H}_2\text{N}\text{C}\text{CH} \quad \text{R}
\]

an amino acid

\[
\begin{array}{ccc}
\text{H}_2\text{N} & \text{C} & \text{O} \\
\text{R} & \text{N} & \text{H} \\
\text{R}_1 & \text{C} & \text{O} \\
\text{R}_2 & \text{N} & \text{H} \\
& \text{C} & \text{O} \\
& \text{R} & \text{OH}
\end{array}
\]

three amino acids linked with peptide bonds

By having different side chains (R1-R20) a huge diversity of enzymes can be made with many different functions. Human cells have some 30,000 different enzymes in them.

Enzymes are of fundamental importance in almost all of the chemical reactions which take place in living organisms.

When digestion occurs, enzymes released into the mouth, stomach, and intestines catalyze or accelerate reactions which result in the breakdown of large food molecules into small 'building block' molecules the body can more readily use.

Four digestive enzyme examples:

- **salivary amylase**: breaks down starch into maltose.
- **gastric pepsin**: breaks down protein into amino acids.
- **pancreatic chymotrypsin**: breaks down protein into amino acids.
- **pancreatic lipase**: breaks down fats into fatty acids.

Enzymes are protein molecules. The enzyme acts on smaller molecules called substrates. Any environmental conditions which destroy protein molecules will also abolish enzymatic activity. For example, egg whites are made up largely of an enzyme named lysozyme, which destroys bacterial cell walls and protects the egg from infection. When we cook the egg white it becomes opaque and hard and the enzymatic activity of lysozyme is destroyed.

**Enzymes**, like salivary amylase, work on a very specific target molecule, or substrate, to produce a product. The relationship between substrates, enzymes and products can be represented by the equation:

\[\text{ENZYME} \rightarrow \text{SUBSTRATE} \rightarrow \text{PRODUCT}\]

The enzyme promotes conversion of the substrate into the product, but is not used up during the reaction; it is a catalyst.

Salivary amylase is a typical human enzyme: it is made up of about 500 amino acid building blocks with a total molecule weight (MW) of about 60,000 (about 170 times more than its product, maltose, MW 360.31).
Approximately one liter of saliva is secreted into the human mouth each day by three pairs of salivary glands. Saliva contains many enzymes, including salivary amylase, the enzyme we will be assaying today.

The substrate for amylase is starch, a polysaccharide (literally "many-sugar"). The product of the amylase reaction is maltose, a disaccharide (literally "two-sugar") made from two glucose molecules. The reaction is summarized below:

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POLYSACCHARIDE + SALIVARY AMYLASE ->
MALTOSE + SALIVARY AMYLASE + smaller polysaccharides
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Our assay takes advantage of the fact that starch (but not maltose) reacts with Iodine (I) to give a dark blue/black solution.

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starch + I -> blue/black complex
starch + Amylase -> maltose
maltose + I -> no color change
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Eventually the enzyme will breakdown all of the starch and the reaction with the iodine will not give the dark iodine/starch complex. How soon this dark color is lost is a measure of the amount (and potency) of the amylase in your saliva.

**HAZARDS**

The Iodine test solution is toxic and caustic and will stain clothing and burn skin. Handle with care, avoid contact with skin or eyes. Wash with lots of water should contact occur.

**PROCEDURE**

**Qualitative assay for salivary amylase:**
1) Note the time
2) Obtain an unsalted cracker from your instructor, place it on your tongue and, without chewing, allow it to dissolve in your saliva. Note the time when (and if) you perceive a faint sweet taste.

Note: amylase activity varies greatly among individuals (some individuals have no amylase activity) thus we will need to be flexible in the next assay (step 9 & 10).

**Semi-Quantitative assay for salivary amylase** (all solutions at room temp)
1) Saliva: Have one partner chew on a clean rubber band and drool into a test tube until you have ~5ml of saliva (Science requires sacrifices from all of us). Note the sex and age of the "spitter"
2) Diluted Saliva: Transfer 1ml of the saliva to a fresh beaker and add 9ml of tap water (NOT DIH₂O) to the saliva and mix well with a pipet until homogeneous.
3) Prepare 8 small test tubes in the white plastic rack labeled #1 to #8
4) Add 1 drop of the Iodine test solution to each tube.
5) Add 1ml of 0.5% starch solution to tube #1 and note the dark blue/black color change.
6) Prepare a small beaker with 20ml of the 0.5% starch solution. Get Ready! You will need to be neat and fast for the next few steps to make the assay work!
7) Note the exact time (T0) and add 1ml of the Diluted saliva (step 2) to the 20ml of starch solution and mix well.
Be ready to immediately remove 1ml of this mixture (use the same pipet) and add it to tube #2 when 30 seconds (T0.5) have passed. Observe the tube immediately on addition of the starch/saliva solution and note color.

8) Continue to remove 1ml amounts from the starch/saliva solution at T1, T2, T4, T8, T16 and T32 minutes, always noting the color change immediately after mixing with the Iodine solution. When the solution no longer darkens you are done; all the starch has been digested by the enzyme. Note which sample was the first to remain light.

9) A) If your solution never went light, your amylase activity was too low to measure in this assay, and you will need to retest a higher concentration of your saliva. Rinse out your tubes several times and dry them upside-down on paper towels, repeat steps 3-8 using 1ml of undiluted saliva (step 1) instead of diluted saliva.

B) If your solution was light from tube #2 on you have SUPER SPIT! Your amylase activity was too high to measure in this assay, and you will need to retest a lower concentration of your saliva. Rinse out your tubes several times and dry them upside-down on paper towels, prepare a further dilution by removing 1ml of the diluted saliva (step 2) and adding it to 9ml of fresh tap water in a new beaker making very dilute saliva, use this to repeat steps 3-8.

10) Compare your results within the class and try to spot trends and associations for different peoples amylase activities. Is amylase activity correlated with age, sex etc?

QUESTIONS

1. What is your groups ranking in the class for amylase activity?

2. The iodine solution contains HCl, a strong acid, specifically because the enzyme, salivary amylase, cannot tolerate acidic conditions. Why was this important? What would happen if the iodine solution wasn't acidic?

3. Since mouth bacteria love sugars, and they produce more acids when sugars are present, could high levels of salivary amylase activity mean more or less tooth decay?

Include the answers to these questions in your lab write up.