ENZYME CATALYSIS

This Experiment was written by Mike Haaf.

An **enzyme** is a biological catalyst. That is, an enzyme enhances the rate of particular biological reactions without being consumed in the process. This rate enhancement is exhibited in figure 1 in which reaction time is measured for an enzyme catalyzed reaction and for the same reaction without the aid of an enzyme. Note that although enzymes increase the rate of a specific reaction relative to a reaction with no enzyme , they don't affect the final concentration of products in the reaction (i.e. the *amount* of reaction products obtained is not dependent on the presence of an enzyme). Without enzymes however, many biological reactions would proceed too slowly to be beneficial to the system.

Enzymes are usually very large protein molecules (MW = 10,000-500,000 g/mol) tailor made with a series of amino acids. All amino acids have the same general structure (shown in figure 2) and differ only in which R group is attached to the central carbon atom.

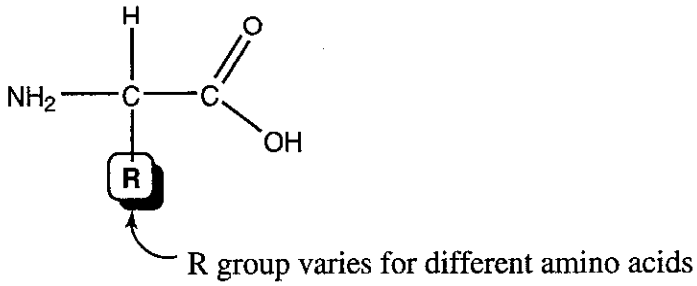


Figure 1: The general structure of an amino acid

The amino acids are linked together via peptide (amide) bonds to form a polymeric material with a very specific amino acid sequence see figure 3).

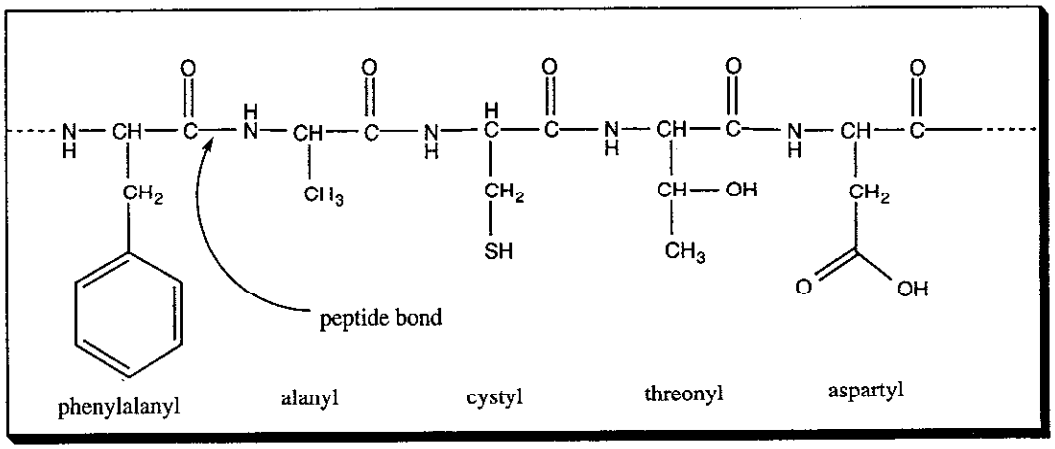
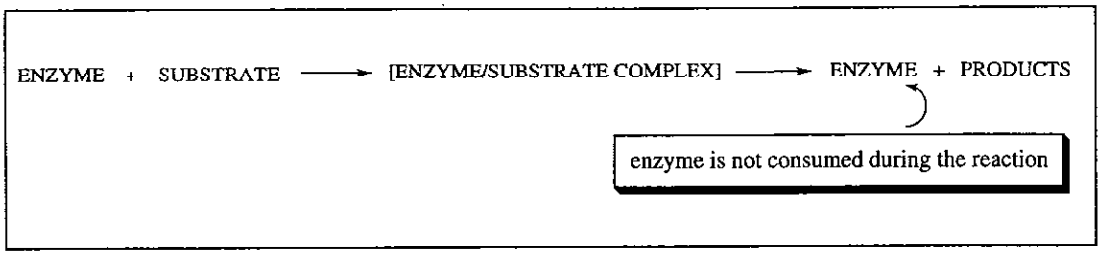


Figure 2: A portion of an amino acid polymer (polypeptide). The amino acids are labeled.

The sequence of amino acids cause the amino acid polymer (polypeptide) to fold up into a very particular three dimensional arrangement. The shape of the enzyme is vital in dictating the chemistry it will facilitate. Each enzyme typically has a reactive cleft or crevice in which a separate chemical species, known as a substrate, can bind. This cleft is called the *active site* of the enzyme and due to its unique shape, is extremely selective in allowing substrates to bind to it. Once a substrate binds to the active site, the enzyme can readily convert the substrate into the

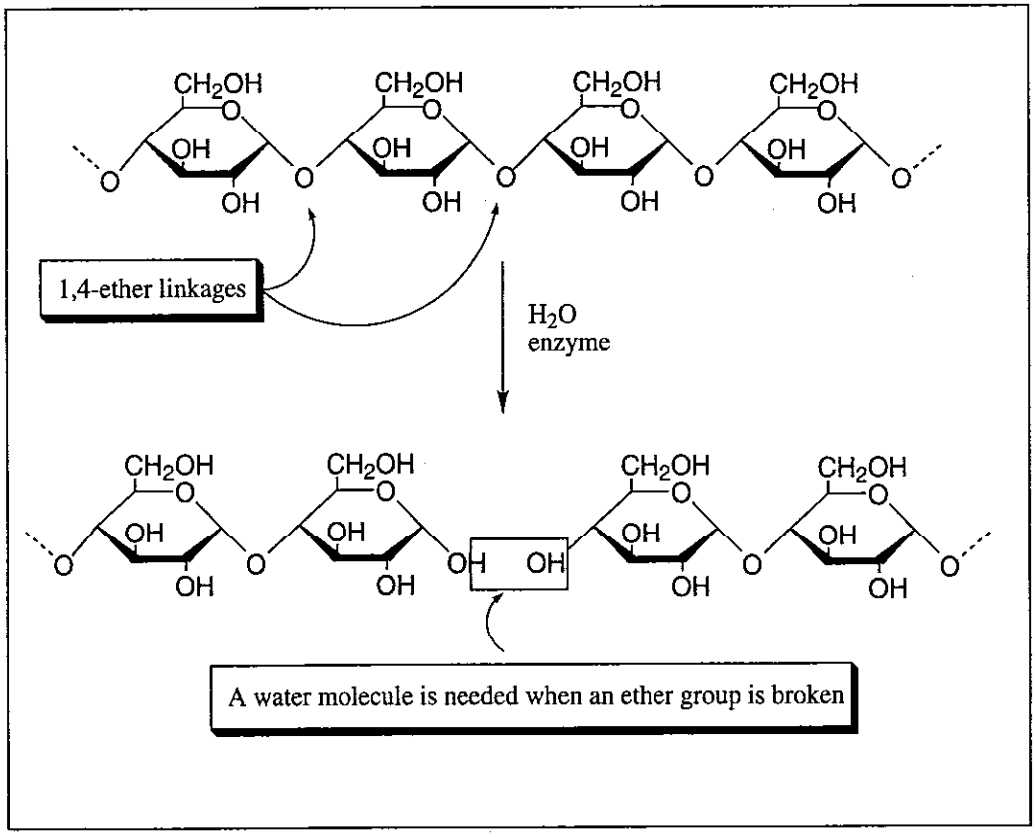
desired products. Since only very specific substrates can bind to a certain enzyme, the enzyme ultimately serves as a catalyst for a very specific biological reaction!! (See Scheme 1)



Scheme 1: Reaction pathway for enzyme catalyzed tranformations.

In addition to being highly selective, enzymes are also highly efficient. Numerous enzymes can convert up to 10,000 substrate molecules to products in one minute! That is, the "turnover number" for many enzymes is approximately 10,000. However, the chemical environment can drastically effect the extremely complex three dimensional structure of the enzyme and thus, can strongly influence the activity of an enzyme. For example. pH, temperature, and enzyme concentration are just a few of the many important factors which effect the activity of an enzyme.

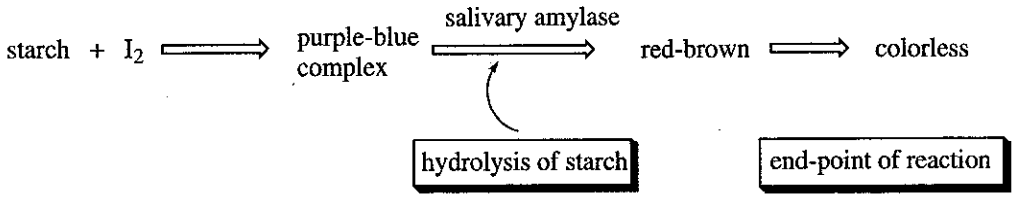
Salivary amylase (MW = 45,000g/mol) is an enzyme responsible for catalyzing the initial breakdown (hydrolysis) of large glucose polymers such as starch or glycogen into smaller units. These are eventually further broken down into their component sugar monomers by other digestive enzymes in the body . The sugars are then used by the body as an important source of energy. The net hydrolysis reaction involves the cleavage of an ether linkage through the addition of a water molecule (see Scheme 1).



Scheme 2: Catalyzed hydrolysis of glucose polymer

The presence of salivary amylase in saliva indicates that digestion of glucose polymers begins immediately after ingestion.

In its high polymer, unhydrolyzed form, starch is capable of forming a purple-blue complex with iodine. Once the starch begins to hydrolyze, the complex breaks down and the purple-blue color turns to red-brown and eventually disappears to give a colorless solution (see Scheme 2).



*Note: The solution may turn colorless without turning red-brown first.

Scheme 3: The starch-I_2 complex and its use as an indicator for enzymatic study.

This is an excellent way of detecting the presence of starch and observing the breakdown of this polymer. Thus, starch is a convenient substrate to use for the study of salivary amylase.

The rate of starch hydrolysis can be determined by measuring the length of time it takes salivary amylase to catalyze the hydrolysis of starch. It is possible to monitor the progress of this reaction with the starch-iodine complex. When the purple-blue color disappears, the hydrolysis reaction of the starch is complete. In this lab, you will use this experimental technique to learn about enzyme kinetics and to witness the effects of temperature and enzyme concentration on the activity of a specific enzyme, salivary amylase.

PROCEDURE

The rate of hydrolysis of a starch sample will be determined by measuring the time it takes for a starch sample (in a starch/iodine complex) to change from a blue-purple color to a colorless solution. The red-brown color may or may not appear during the hydrolysis. The rate of starch breakdown will be determined for several different temperatures and for a variety of enzyme concentrations. YOU SHOULD WORK IN PAIRS.

A. Effect of Enzyme Concentration on Reaction Rate

- Collect approximately 2mL of saliva in a small beaker. This saliva solution will serve as your "stock" solution.
  NOTE: You should avoid chewing gum, eating candy, or drinking liquids with a lot of sugar for about an hour before this lab!
  BOTH STUDENTS IN EACH PAIR SHOULD COLLECT SALIVA IN THE CASE THAT ONE SAMPLE DOES NOT WORK!
- Obtain a porcelain spot plate. Prepare the plate by adding one drop of iodine solution to each cup on the plate. To the last spot, add two drops of end point indicator to serve as a reference for ensuing experiments.
- Prepare a series of 4 clean, dry test tubes and pipette 1.0mL of starch solution into each tube. These solutions will be used later.
- Prepare a series of test tubes containing 1:10, 1:20, 1:40, and 1:80 dilutions of saliva in water.

Prepare the 1:10 dilution by pipetting 0.5mL of saliva (using a 1.0mL pipette) into 4.5mL of distilled water. Mix thoroughly.

- Immediately after mixing the saliva solution, transfer 1.0mL of the saliva solution into one of the test tubes containing starch solution. Mix this combination to assure even distribution of enzyme and substrate.
- RECORD THE TIME THESE SOLUTIONS WERE MIXED!! Then, at 30 second intervals, remove a drop of the starch-enzyme mixture and add it to a spot of iodine solution on the porcelain spot plate. Continue this every minute until the starch-enzyme solutions reach the end-point color (red-brown or colorless).
  NOTE: Add the starch-enzyme drops to a *different iodine spot* each time.
  NOTE: Careful observations are especially important here.
- Prepare the 1:20 dilution in another test tube by pipetting 0.5mL of stock saliva into 9.5mL of distilled water. Mix thoroughly.
- Add 1.0mL of the1:20 dilution to a second starch solution and perform the assay described for the 1:10 dilution. Record the time it took to reach the end point.
- Prepare the 1:40 dilution; assay as described above.
- Prepare the 1:80 dilution; assay as described above.

B. Effect of Temperature upon an Enzymatic Reaction

- Prepare a series of large water baths in 250mL or 400mL beakers. The baths should be as follows:
  - 5°C (ice/water)
  - 20°C (cold tap water)
  - 40°C (warm tap water)
  - 100°C (boiling water)
- Fill 4 test tubes each with 1mL of starch solution and place one in each water bath. Allow these to sit in the baths for at least one minute so the solution has time to come to temperature.
- Add 1mL of FRESH 1:20 dilution of saliva to each starch solution and mix thoroughly.
- Assay these mixtures to test how well they form the iodine complex. Test each tube every 2 minutes. Stagger the testing times for each tube so you can be testing samples every 30 seconds.
- Record the total time it took for each reaction to reach the endpoint.

C. Graphical Analysis

- Prepare a graph relating reaction rate (length of time needed to reach endpoint) and enzyme concentration. Do you notice a relationship??
- Make a second graph relating reaction rate and temperature. What conclusions can you draw from your data?