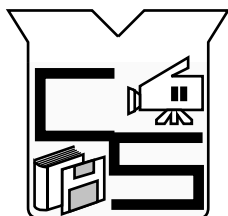
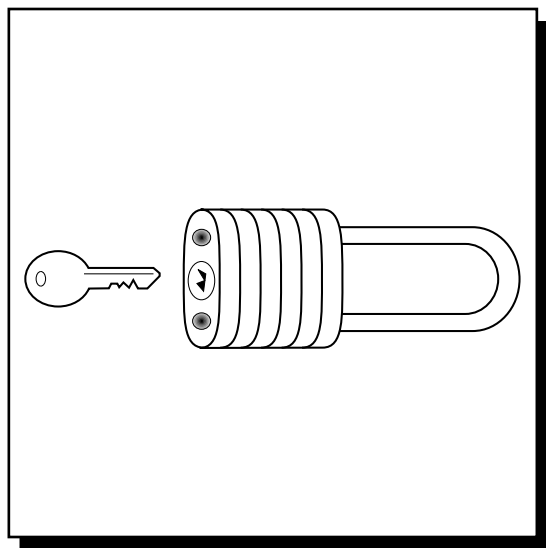


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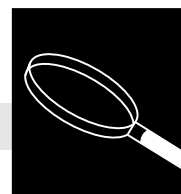


ChemSource

*Instructional Resources for Preservice and
Inservice Chemistry Teachers*

**ENZYMES: BIOCHEMICAL
CATALYSTS**

Topic Overview



CONTENT IN A NUTSHELL

In order to develop, grow, and reproduce, biological organisms depend upon thousands of chemical reactions. These reactions are called *biochemical reactions* and they occur because of specialized catalysts called enzymes. Although these enzymes do not force a reaction to occur that would not occur otherwise, they greatly increase the rates of body reactions that allow us to produce the energy we need to survive. If it were not for these biochemical catalysts, our body reactions would be so slow that life would be impossible.

Over 2,000 different enzymes in the body are known. This large number is necessary because enzymes are very particular about the reactions they catalyze; in fact, an enzyme may catalyze only one specific reaction. Enzymes are present in every body cell. One enzyme, which we will study in this module, is the enzyme *catalase*. It is produced in small organelles called *peroxisomes*, or *microbodies*. The enzyme catalase breaks down harmful hydrogen peroxide when it is produced excessively by body cells.

All enzymes are protein molecules. Although very large protein molecules, there is one primary area of the enzyme molecule where it chemically reacts with a substance called a **substrate**. This part of the molecule is called the **active site**. We can understand how an enzyme works by comparing its action on a substrate molecule to that of a key in a lock. The ridges and grooves of the key represent the active site of the enzyme, and, just as each key fits only a particular lock, each enzyme fits only a particular substrate. Also, just as the key opens the lock and is not destroyed in the process, enzymes—like other catalysts—emerge intact from a biochemical reaction.

Sometimes other molecules block enzymes or otherwise interfere with their reaction at the active site. These species include certain metal ions, insecticides, poisons, and bacterial toxins (like botulin that produces botulism). This process is called **enzyme inhibition**.

Enzymes act on substrates at a very rapid rate. One *catalase* enzyme molecule, for example, will completely break down 5.6 *million* hydrogen peroxide molecules *per minute*. Catalase is considered a relatively slow enzyme! One of the fastest enzymes, carbonic anhydrase, will break down 36 million carbonic acid molecules per minute. Now that's fast!

Since enzymes are protein molecules, they have the properties of proteins. They are denatured (rendered inactive) by high temperatures and many by extremes in pH. As the temperature of an enzyme-catalyzed reaction increases, the rate increases until the temperature is high enough to denature the protein.

PLACE IN THE CURRICULUM

This is an enrichment topic. It is most appropriate for the student who has had a general chemistry course.

CENTRAL CONCEPTS

1. Enzymes are special molecules that catalyze biochemical reactions.
2. All enzymes are protein molecules, and have general properties of proteins, *i.e.*, they are altered by pH and temperature changes.

3. Usually an enzyme acts only on a specific molecule (substrate), by first forming an enzyme-substrate complex, then breaking down into products and regenerating the enzyme.
4. Enzymes must be activated before they have the ability to function as a catalyst.
5. Only a small part of the enzyme molecule acts on the substrate. This part of the enzyme is referred to as the **active site**.
6. Enzymes can be inhibited when other substances block the active site.

1. pH
2. Chemical kinetics, especially the effects of temperature on reaction rates (see *Reaction Rates* module)
3. Characteristics of proteins
4. Structure of proteins
5. Denaturation of proteins

RELATED CONCEPTS

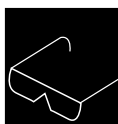
1. Collect and plot data on a graph.
2. Interpret a graph.
3. Measure temperature.
4. Work with general laboratory glassware.
5. Set up apparatus to collect gas by displacement of water.

RELATED SKILLS

Concept/Skills Development



LABORATORY ACTIVITY: STUDENT VERSION



Activity 1: The Effects of Temperature and Inhibitor on the Enzyme Catalase Extracted from Potato

Introduction

Catalase is an enzyme found in fairly high concentration in several fruits and vegetables. Its specific action is to decompose hydrogen peroxide into water and oxygen gas. This enzyme is also found in animal blood, and accounts for the “fizzing” when hydrogen peroxide is placed on an open wound. When the temperature is increased, the activity of enzymes also increases. However, a temperature is eventually reached where the protein enzyme is denatured and is no longer active. Enzymes can be inhibited by many substances. Catalase is inhibited by the copper(II) ion.

Purpose

To show that an enzyme can be isolated from a simple source, a potato, and that the activity of the enzyme depends upon temperature. To show that copper(II) ion is an inhibitor for the enzyme catalase.

Safety

1. Wear protective goggles throughout the laboratory activity.
2. Observe general safety rules when conducting this activity. None of the materials or solutions pose unusual safety hazards or require special handling.

NOTE: There are two alternate procedures for this activity. Your teacher will tell you which one to do.

Procedure A: Measuring Height of Foam Produced by Catalase

Procedure

1. Place potato pulp in a 50-mL graduated cylinder to a depth of about 3 cm.
2. Add 10 mL 3% hydrogen peroxide. Stir the cylinder quickly to mix the pulp and the hydrogen peroxide.
3. Note the time that a foam begins to form.
4. After 1 min, measure the height of the foam (from the top of the liquid to the top of the foam)
5. Measure and record the foam height each minute for at least 5 min.
6. Construct a graph that will allow you to record foam height on the *y*-axis and time on the *x*-axis. Label this *Graph 1*.

Effect of Temperature on Foam Height

1. Add potato pulp to a depth of about 3 cm to a second graduated cylinder and place the cylinder in a water bath for 2 to 3 min. *Your teacher will specify the temperature for your water bath.* Record the temperature.
2. Add 10 mL 3% hydrogen peroxide to another graduated cylinder.
3. Place both cylinders in a water bath for 5 min. *Your teacher will specify the temperature for your water bath.*
4. Remove both cylinders, quickly pour the 3% hydrogen peroxide into the potato pulp. Put the cylinder back into the water bath. Measure the foam height after 5 min and record your data.

Effect of an Inhibitor on Foam Height

1. Add potato pulp to a depth of about 3 cm to a fourth graduated cylinder. Add 10 drops copper(II) sulfate solution to the cylinder and stir.
2. Place 10 mL 3% hydrogen peroxide in a separate graduated cylinder.
3. Place both cylinders in a water bath at 20 °C for about 5 min.
4. Remove the cylinders from the water bath and add the 3% hydrogen peroxide to the potato pulp mixture.
5. Measure the foam height after 5 min. How does the foam height compare to that produced above without the copper(II) sulfate added?
6. Thoroughly wash your hands before leaving the laboratory.

Data Analysis

1. According to the data you plotted on *Graph 1*, how does the quantity of foam produced by the breakdown of hydrogen peroxide vary with time?
2. Your teacher will ask you to plot your data for temperature vs. height of foam on a master graph along with the data from your classmates.

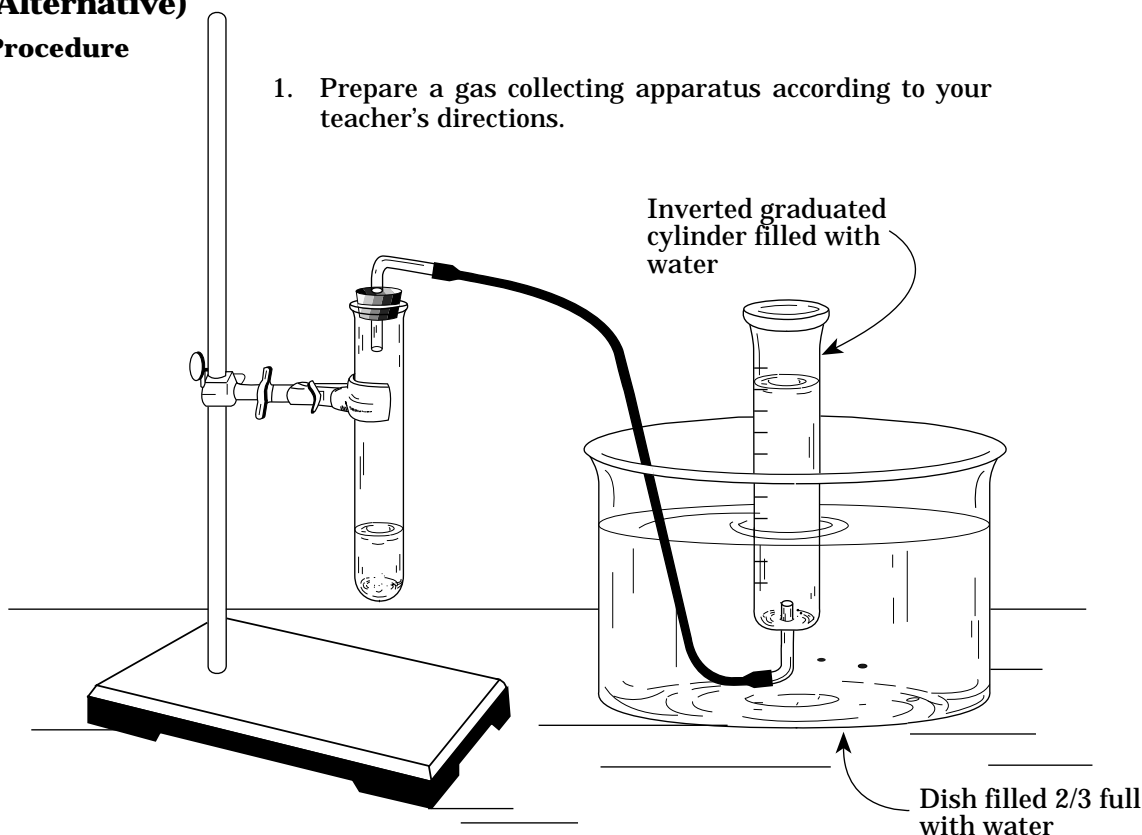
According to data plotted on the master graph, what is the effect of temperature on the rate of enzymatic action? At some point, did the quantity of foam *not* increase as temperature increased? How can you account for this?

3. According to your data, what effect did adding an inhibitor [copper(II) ions] have on the rate of foam production? Did the production of foam cease completely? How can you account for this?

Procedure B: Measuring Volume of Oxygen Produced by Catalase (Alternative)

Procedure

1. Prepare a gas collecting apparatus according to your teacher's directions.





2. Add about 10 mL 3% hydrogen peroxide to a small test-tube.
3. Quickly add 5 mL catalase extract (from the potato pulp).
4. Stopper the test-tube, swirl it once to mix the contents, and place the tubing leading from the stopper beneath an inverted, water-filled graduated cylinder. Record the time.
5. Record the volume of gas produced each minute for 5 min. Record your data.
6. Construct a graph that will allow you to record volume of gas produced on the *y*-axis and time on the *x*-axis. Label this *Graph 1*.

Effect of Temperature on Volume of Gas Produced

1. Add 10 mL catalase extract to a small test-tube.
2. Add 10 mL 3% hydrogen peroxide to a second small test-tube.
3. Place both test-tubes in a water bath for 5 min. *Your teacher will specify the temperature for your water bath.*
4. Remove both tubes, quickly pour 3% hydrogen peroxide into the catalase, stopper, and collect the gas produced as in the procedure above.
5. After 5 min, measure the amount of gas produced and record your data.

Effect of an Inhibitor on the Volume of Gas Produced

1. Place 10 mL catalase extract in a small test-tube.
2. Add 2 mL copper(II) sulfate solution to the tube.
3. Place 10 mL 3% hydrogen peroxide in a separate tube.
4. Place both tubes in a water bath at 20 °C for about 5 min.
5. Remove the tubes, add the 3% hydrogen peroxide to the catalase, shake to mix.
6. Quickly stopper the tube and collect the gas as you did above.
7. Measure the volume of gas produced after 5 min and record your data.
8. Thoroughly wash your hands before leaving the laboratory.

Data Analysis

1. According to the data you plotted on *Graph 1*, how does the volume of gas produced by the breakdown of hydrogen peroxide vary with time?
2. Your teacher will ask you to plot your data for temperature vs. volume of gas on a master graph along with the data from your classmates.

According to the data plotted on the master graph, what is the effect of temperature on the rate of enzymatic action? At some point, did the volume of gas *not* increase as temperature increased? How can you account for this?

3. According to your data, what effect did adding an inhibitor [copper(II) ions] have on the rate of gas production? Did the production of gas cease completely? How can you account for this?

Implications and Applications

1. Enzymes have maximum activity at a temperature that depends upon the environment in which the organism thrives. Almost all enzymes found in the human body have maximum activity at 37 °C (98.6 °F). Can you explain this?
2. Most enzymes are inactivated at temperatures above 60 °C. What does this tell you about the effect of heat on body functions?
3. Increasing the temperature will increase the rate of reaction. In fact, it has been estimated that increasing the temperature by 10 °C will double the rate of enzymatic reactions. Do your data support this assumption?
4. Enzymes are typically found and used around the house. For example, meat tenderizer contains a *proteinase* that helps break down fiber and makes meat more tender. Would you add meat tenderizer to a steak while it is on the grill cooking? Some detergents contain a proteinase enzyme to help remove grime and stains, which are largely protein. Do you get best results from these detergents in *cold* or *warm* water?
5. Just as the copper ion inhibited the action of the enzyme catalase, other metal ions inhibit other specific enzymes. For example, mercury, lead, beryllium, and arsenic inhibit specific enzymes required for energy production. It is primarily for this reason that these metals are considered *toxic* and must be used with caution.



**LABORATORY
ACTIVITY:
TEACHER
NOTES**

***Activity 1: The Effects of Temperature and Inhibitor
on the Enzyme Catalase Extracted from Potato***

Major Chemical Concept

Enzymes are proteins that act as catalysts for biochemical reactions. The rate at which the catalyzed reaction occurs varies with temperature until the temperature is reached where the protein catalyst is deactivated. Catalysts are also inhibited by certain substances, including certain metal ions.

Level

This material is appropriate for first year or advanced high school chemistry classes.

Time

40-45 min

Expected Student Background

Students should understand the role of catalysts in a chemical reaction, the nature of proteins, and protein structure. They must be able to collect data, prepare a graph, interpret the graph, and make predictions. If students use *Procedure B*, they should know how to collect oxygen gas when it is produced in a chemical reaction.

Safety

Read the *Safety Considerations* in the *Student Version*. Materials used in these activities pose few safety problems. Students should not eat the potatoes used in the activity. Care should be taken with the hydrogen peroxide and copper(II) sulfate solutions. Wash with water if any gets on the skin.

Materials (For 24 students working in pairs)

- Potatoes (red-skinned or Irish potatoes work well; see *Advance Preparation*)
- 0.1 M Copper(II) sulfate solution, CuSO_4 (see *Advance Preparation*)
- Fresh 3% hydrogen peroxide, H_2O_2 (from drug or grocery store), 600 mL
- 60 Graduated cylinders, 50-mL
- 60 10- x 150-mm test tubes with stoppers
- 12 Metric rulers
- 4 Water baths (large beakers may be used)
- Clock with second hand.
- 12 Graduated cylinders, 10-mL
- 12 Thermometers
- 4 Hot plates or burners
- 12 Gas collecting materials (for *Procedure B*): one-hole stopper (to fit test-tube) with glass tubing (or plastic tubing) through hole; flexible hose attached to the glass tube, leading to water trough filled 2/3 with water and water-filled inverted
- 12 Graduated cylinders, 100-mL

Advance Preparation

0.1 M Copper(II) sulfate solution: Dissolve 2.5 g copper(II) sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in 100 mL water.

Potato extract. Prepare the pulp and extract as follows: Peel 2-3 Irish potatoes. Cut the potatoes into pieces and place them in a blender or food processor. Add about 50 mL water and thoroughly grind the potatoes. Let the mixture stand for a few minutes, and filter it through cheesecloth to obtain a pulp-free solution. The enzyme catalase is soluble, so it is in both the pulp and the liquid extract.

Drug store variety hydrogen peroxide is 3%. *It must be fresh!*

Pre-Laboratory Discussion

1. Review graphing.
2. Discuss general properties of proteins.
3. Discuss general principles of reaction kinetics (see *Reaction Rates* module).
4. Assign *Procedure A* or *Procedure B* (or both).
5. Assign temperature to groups. Recommended temperatures are:
Approximately 0 °C (ice bath)
Room Temperature, 20-25 °C
Body Temperature, 37-40 °C
High Temperature, 70-80 °C
6. Explain the nature of a *foam* (a substance that has trapped a gas).

Teacher-Student Interaction

The success of this activity requires a *group* effort. Students must be encouraged to contribute their data, even if it does not seem to *fit* a pattern on the group graph. It is imperative that you move from group to group to be sure that they are doing the activities correctly and safely. Question students about their results as they are conducting the activities.

Anticipated Student Results and Answers to Data Analysis

1. Volume of gas (foam height) vs. time: The volume of gas (foam height) will increase regularly, but may tend to level off after 5 min or so.
2. Temperature vs. volume of gas (foam height): The volume of gas (foam height) will increase as the temperature increases, but will drop off at higher temperatures.
3. Inhibitor and enzyme: The volume of gas (foam height) will be much less, but may not drop to zero.

Answers to Implications and Applications

1. This is normal body temperature. As cells evolved, they adapted to the temperature that would produce maximum product from enzyme action.
2. High body temperature can inactivate protein, including the protein component of enzyme systems.
3. Yes. Class data will generally show a doubling of reaction rate with each 10°C increase in temperature.
4. You would not add meat tenderizer to meat while it was cooking, since the thermal energy would inactivate the enzymes in the meat tenderizer. The best results would be in warm water. This would increase the activity of the enzymes. If the water were too hot, however, the enzymes would become inactivated.

Post-Laboratory Discussion

1. If data seem to be “nonfitting” to graph, discuss experimental sources of error. Ask students to suggest possible sources of error.
2. Discuss the general relationship between rates of reactions and temperature.
3. Discuss the evidence for enzyme inhibition and gas production.
4. Ask students to suggest applications.



Possible Extension

1. Try other simple enzymes. Refer to college biochemistry texts for examples.
2. pH also affects enzymes. Design studies to show the effect of pH on catalase and other enzymes.
3. Have students perform library research and write reports pertaining to enzymes.

Assessing Laboratory Learning

Although you should not penalize students for not obtaining data that “fit” the graphs, they should be evaluated on their laboratory technique, ability to work as a group, and ability to record data.

References

These activities are modified from those found in:

Borgford, C., and Summerlin, L. (1988). *Chemical activities*. Washington: American Chemical Society.

Summerlin, L., Borgford, C., and Ealy, J. (1987). *Chemical demonstrations: A sourcebook for teachers, Volume 2*. Washington: American Chemical Society.

DEMONSTRATIONS

CAUTION: Use appropriate safety guidelines in performing demonstrations.

Demonstration 1: Calcium ions, Rennin, and the Coagulation of Milk

Purpose

This demonstration illustrates the importance of a metal ion as an enzyme activator.

Safety

There are no special precautions for this demonstration. Students should be careful with the warm water.

Materials

Whole milk

Sodium citrate

Rennin (sold in grocery stores as Rennilase or Rennet), available from biological supply companies

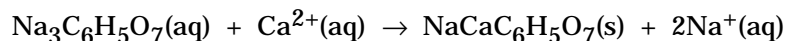
Beaker of warm water

Procedure

Place 15-mL samples of fresh, whole milk in each of two large test-tubes. To one, add a pinch (match-head size) of sodium citrate, and stir. Add nothing to the other test-tube. Add a small amount of the enzyme to both test-tubes (about 5 drops of rennilase, or about 1/4 tablet of rennet). Place both test-tubes in a larger beaker of warm water for three to 5 min. Compare the results.

Remarks

Rennin is an enzyme that converts the soluble milk protein caseinogen into the insoluble protein paracasein, producing the curd that can be processed into cheese or other milk products. Calcium ion is required to activate the enzyme rennin. In this demonstration, calcium ion is removed by precipitating it as calcium citrate. In the tube containing the sodium citrate, calcium citrate forms as a precipitate:



In the absence of calcium ions, the rennin is not activated and cannot catalyze the reaction to convert caseinogen into paracasein; thus, the milk does not coagulate.

Reference

Borgford, C., and Summerlin, L. (1988). *Chemical activities*. Washington, DC: American Chemical Society.

Demonstration 2: Pectinase and Apple Juice Production

Purpose

The activity of a typical enzyme, pectinase, is illustrated in this demonstration. Pectinase breaks the cell wall of fruit, producing more “juice.”

Safety

Students should not eat or drink any of the material from this demonstration.

Materials

Applesauce
Pectinase enzyme (available from chemical or biological supply companies)
Beakers
Droppers
Graduated cylinder
Filter paper

Procedure

Measure about 50 mL applesauce into each of two small beakers. Add 1-mL pectinase to the applesauce in one beaker; add nothing to the other beaker. Stir the applesauce in both beakers, and allow to stand for 10 min. Filter each, and measure the amount of juice produced from the applesauce with and without the enzyme.

Remarks

Pectin is a large polysaccharide that is present in fruit cells. It prevents material, including the juice of the fruit, from settling out in the cells. When this large molecule is acted on by the enzyme pectinase, the juice can be easily separated. The pectinase enzyme ruptures the cells of the apple, allowing the juice to settle and to be easily separated from the other material in the cell.

Extensions

Students should be encouraged to devise activities to answer the following questions:

1. What is the relationship between the amount of enzyme added and the amount of juice produced? [*The greater amounts of enzyme will lead to production of more juice.*]
2. What is the relationship between time of standing and the amount of juice? [*Typically more juice will be produced.*]
3. What is the relationship between the temperature and the amount of juice produced? [*Enzyme activity is dependent on the temperature.*]

Reference

Borgford, C., and Summerlin, L. (1988). *Chemical activities*. Washington, DC: American Chemical Society.



Demonstration 3: Catalase in Turnips, Potatoes, and Rutabagas

Purpose

To demonstrate the activity of a typical enzyme.

Safety

Be careful when slicing the vegetables in the demonstration.

Materials

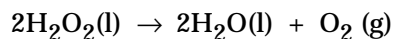
3% Hydrogen peroxide (drug store variety)
Potatoes, turnips, rutabagas—fresh, whole; animal tissue, such as liver, works well

Procedure

Slice several potatoes, turnips, and rutabagas into 3-4 smaller pieces. Place each sample in a separate shallow dish. Pour sufficient 3% hydrogen peroxide to cover the exposed surface. Pass the dishes around to allow students to observe the bubbling on the cut surfaces of the vegetables. You might observe cut side vs. skin side of vegetables.

Remarks

Catalase, found naturally in the vegetables used here, reacts with the hydrogen peroxide to rapidly decompose it into water and oxygen gas.



Catalase is the enzyme that specifically decomposes hydrogen peroxide into water and oxygen gas. Perhaps you have noticed that when hydrogen peroxide is placed on an open wound, fizzing and bubbling occur. This reaction is caused by catalase in the blood decomposing the hydrogen peroxide and releasing oxygen gas.

Reference

Summerlin, L. (1979). *Chemistry of common substances*. Morristown, NJ: Silver-Burdett.

GROUP AND DISCUSSION ACTIVITIES

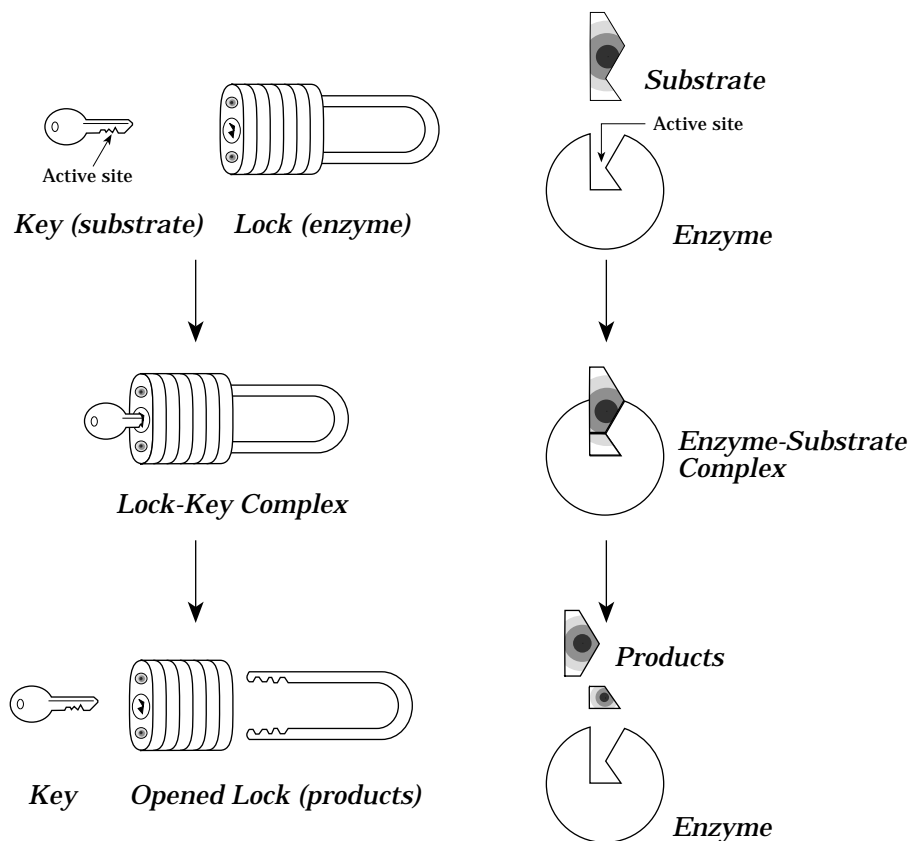
Counterintuitive Example

Proteins (as in meats) are broken down by enzymes produced in the pancreas. The pancreas is composed of protein. Why don't these enzymes digest the pancreas? *[Enzymes, including those produced by the pancreas, are produced as inactive enzymes, or pro-enzymes. When they get to the site where they are needed, they are then "activated" by another substance. The pancreas produces inactive trypsinogen, which is activated in the small intestine by the "activator" secreted by the cells in the wall of the small intestine. The "active" enzyme is trypsin.]*

Metaphors and Analogies

1. Enzymes are usually confined to the area where they are needed to catalyze specific reactions. These areas include the liver, pancreas, and muscle (including heart). When they are found in significant concentration outside those areas, it means that the tissue or organ containing those enzymes has been damaged, allowing them to "escape." This phenomenon can be compared to a prison, where felons are grouped together. If these convicts are seen outside the prison it is cause of great concern!
2. Enzymes bringing together substrate molecules to accelerate a reaction can be compared to a chaperon (enzyme) at a dance bringing bashful students (substrates) together and getting them to know each other (form products).

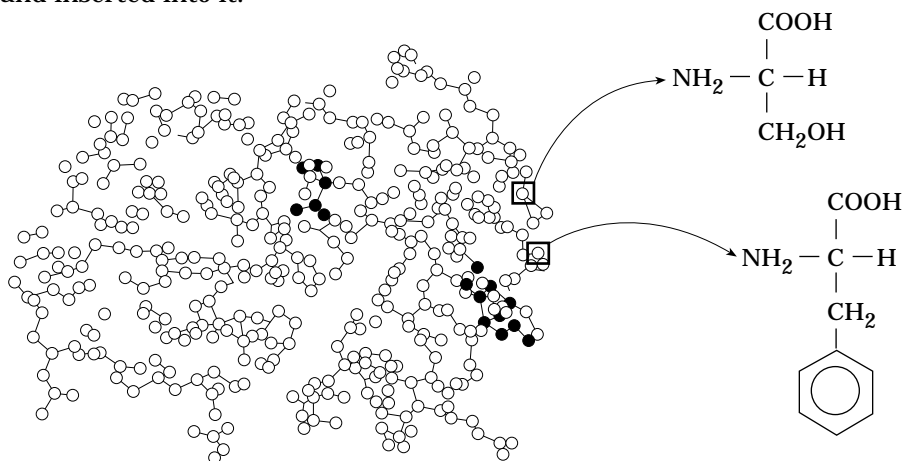
3. The mechanism of enzyme action is well illustrated by the “lock and key” analogy. The “lock” represents the substrate and the “key” represents the enzyme. Just as a key is specific for a certain lock, an enzyme is specific for a certain substrate. Also, just as the enzyme has an “active site” where the chemistry occurs when it combines with a substrate, the key has grooves and notches that must match those in the lock mechanism. After the key “opens” the lock remains intact, just as the enzyme reappears intact after acting on a substrate.



4. A new theory of enzyme activity is the “induced fit” model, where the enzyme conformation changes to accommodate the substrate. This is somewhat like a glove changing to fit the hand inserted into it.

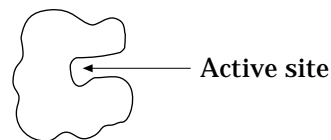
Pictures in the Mind

1. Draw a picture of an enzyme molecule. Each circle represents an amino acid. Typical amino acids are shown here.



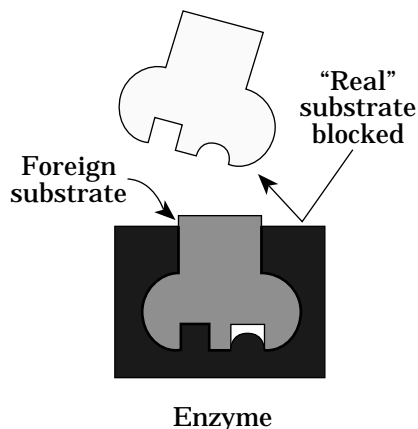
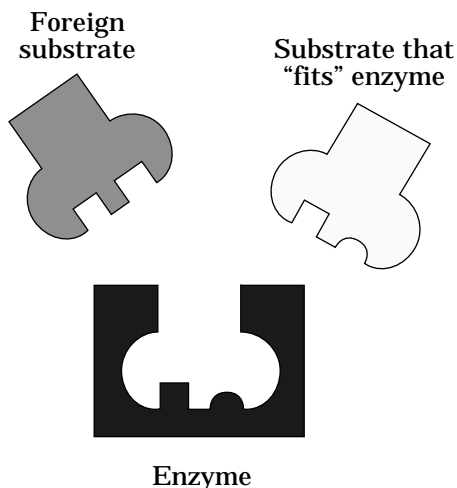


2. Draw a picture of an enzyme molecule showing its *active site*.
3. Draw a picture of a substrate molecule that “fits” the active site of an enzyme, and one that does not “fit.”



FITS

DOES NOT FIT



Pattern Recognition

1. Enzymes were previously named by adding the suffix *-ase* to the name of the substrate upon which they acted. For example:
 - *Sucrase* is the enzyme that acts to break down *sucrose*.
 - *Lipase* breaks down *lipids*.
 - *Peroxidase* breaks down *peroxides*.
2. Enzymes are now properly classified according to the *type* reaction they catalyze. The classes include:
 - *Oxido-Reductases* involve donating and accepting electrons.
 - *Hydrolases* add water to break chemical bonds. (Most digestive enzymes are in this category.)
 - *Transferase* transfers groups (amino, phosphate, carboxyl, *etc.*) from one molecule to another.
 - *Lyases* break (or form) double bonds.
 - *Isomerases* change one isomer into another.
 - *Ligases* allow C—C, C—N, and C—S bond formation.

Problem Solving/Decision Making

1. Humans cannot digest wood, paper, and other cellulose materials because cellulase, the enzyme required to break these materials down into usable glucose, is not present in the body. It is produced by bacteria that live in the intestinal tracts of termites, horses, goats, *etc.* Suppose that someone suddenly found a way for this enzyme to exist in humans. Reflect on the wide implications of such a possibility, and write a short paper describing what effect this might have on humans.



5. What is the relationship between the water-soluble vitamins and enzyme function? Find out which vitamins are related to which enzymes and which disease or deficiency state results when that vitamin is missing. You can research this problem in the library. Ask a physician or pharmacist for advice.

TIPS FOR THE TEACHER

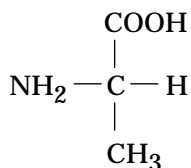
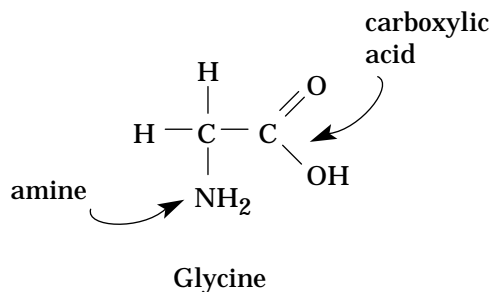
Enzymes are *proteins*. The word *protein* is from the Greek *protos*, meaning “first,” or “of prime importance.” This very important group of biochemical molecules are, indeed, of prime importance. They are found in every living cell and they are extremely diverse in form and function. No other biochemical substance serves so many different functions as proteins. Proteins also provide the primary structural component of the cell and also direct the synthesis of cellular materials. Proteins are responsible for transporting materials in the body, including oxygen, ions, lipids, and other substances to the cells where they are needed for metabolism.

All proteins share common features. They are very large molecules with molar masses ranging from 5,000 to several million daltons. Such molecules are often called *macromolecules*. In addition to containing carbon, oxygen, and hydrogen, all proteins contain nitrogen and some contain sulfur. When proteins are consumed as nutrients, it is necessary to remove the nitrogen and excrete it. Some animals excrete nitrogen as ammonia, but ammonia is toxic to humans and nitrogen is excreted as the compound called *urea*. The most important feature of proteins is that they are all polymers of smaller units called *amino acids*.

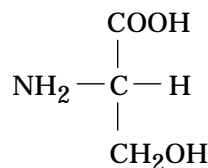
Amino acids are compounds containing both an *aminogroup* and a *carboxylic acid* group. All amino acids found in proteins have these two groups on the same carbon atom.

Of 150 known amino acids, only about 20 commonly occur in human protein. Eight of these common amino acids cannot be synthesized by the body and must be included in the diet. These are called

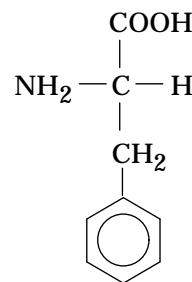
essential amino acids. All of these amino acids have the basic structure as shown, and differ only in the group attached to the carbon atom bearing the amino and carboxylic acid groups. Natural amino acids have the L-configuration. Three typical amino acids are:



L-Alanine

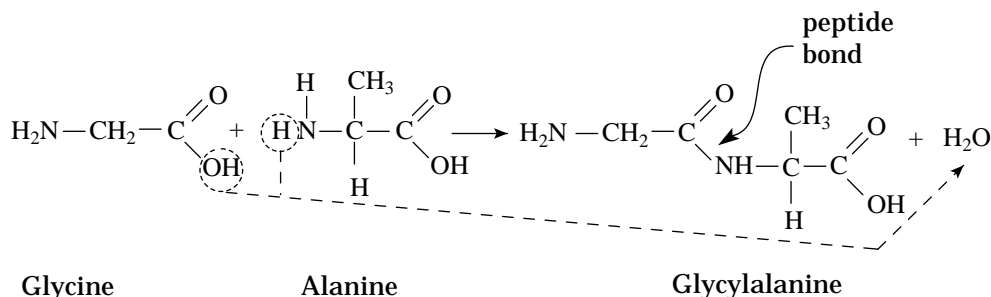


L-Serine

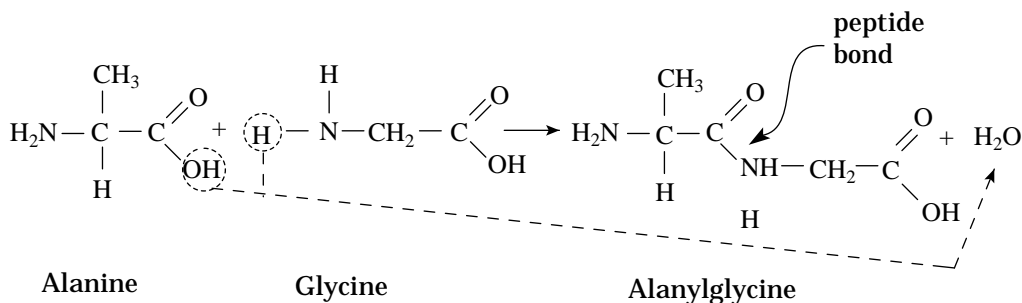


L-Phenylalanine

Amino acids can combine easily by removing a water molecule, joining the amino end of one amino acid with the carboxyl end of another. The resulting bond is called a **peptide bond**. The process of peptide bond formation is identical to the process of condensation polymerization forming nylon (see *Polymers* module). For example, the amino acids glycine and alanine can combine to form a dipeptide, glycylalanine:



When several amino acids are joined in such a manner, the resulting structure is called a **peptide**. Examining the way the peptide bond is formed, it is obvious that it could form by connecting the amino and carboxyl end of either amino acid. Thus, there could be two different ways to join glycine and alanine. For example,



Although glycylalanine and alanylglycine, contain the same two amino acids, they are *two completely different compounds*. In fact, if 4 different amino acids are combined, and if each amino acid can appear more than one time in this sequence of four, it would be possible to form *256 different compounds*. How many different compounds can be formed from all 20 amino acids? A quick calculation shows that 10^{26} compounds are possible! This mind-boggling number clearly shows the possibility of billions of different proteins, all consisting of only 20 amino acids! The order in which these amino acids are strung together is the protein's **primary structure**. When proteins are digested the process is reversed and peptide bonds are broken to release amino acids. Since water is required, the process is called **hydrolysis**.

As polypeptides get larger, cross-linking occurs. An important cross-linking bond is the *disulfide bond*, which is formed by thiols ($-SH$ groups). Thiols can react with heavy metals, such as mercury and lead, which results in the alteration of protein structure. This structural alteration accounts for heavy metal toxicity. As the protein molecule becomes more complex, other bonds—*ionic, hydrogen, and hydrophobic/hydrophilic*—become important in holding the giant molecule together. We can effectively break these bonds and denature or destroy the protein using such things as heat, acids and bases, alcohol, and detergents. Perhaps you can think of some practical examples of protein denaturation (*e.g.*, frying or whipping egg white).

peptide bond bond formed between the carboxylic acid group of one amino acid and the amino group of another amino acid.

primary protein structure sequence of amino acids in a polypeptide.

protein polymer linked together by peptide bonds that gives amino acids upon hydrolysis. Proteins are found in plant and animal tissue.

secondary protein structure orientation of a peptide chain in space.

zwitterion molecule bearing both a positive and a negative charge. Neutral protein molecules usually exist as zwitterions where the acidic carboxylate hydrogen ($-\text{COOH}$) is transferred to the amino group ($-\text{NH}_2$) resulting in the presence of $-\text{COO}^-$ and $-\text{NH}_3^+$ groups in the protein.

Enzymes

apoenzyme inactive, protein component of a holoenzyme.

coenzyme activator that converts an apoenzyme into an active enzyme.

enzyme biocatalyst protein produced by living cells; enzymes regulate cellular reactions without themselves being altered or destroyed.

holoenzyme complete, activated enzyme.

metalloenzyme holoenzyme (active enzyme) produced by an apoenzyme and a metal-ion activator.

molecular rate number of substrate molecules acted upon by a single enzyme molecule per minute.

phenylketonuria build-up of phenylpyruvate in the body due to the absence of the enzyme phenylalanine hydroxylase.

zymogen (also often called **proenzyme**) inactive form of an enzyme.

Common Student Misconceptions

1. “Enzymes *cause* a reaction to occur.”

Enzymes (or other catalysts) will not cause a reaction to “go” if that reaction won’t “go.” However, the enzyme does lower the potential energy barrier (activation energy) and permits the reaction to proceed at a much more rapid rate.

2. “Enzymes (as found in dietary supplements, over-the-counter drugs, etc.) are effective when ingested.”

Enzymes (and other proteins) are degraded in the stomach or intestine and are not absorbed. Only a few digestive enzymes are taken orally, and their effectiveness is questionable.

3. “Only a few enzymes are needed in the body.”

There are over 2,000 *known* biochemical reactions that require specific enzymes; thus there are over 2,000 different enzymes.

4. “Enzymes are found only in animal cells.”

Enzymes are found in both plants and animals, although they are all proteins. Turnips and potatoes, for example, contain the enzyme catalase that breaks down hydrogen peroxide into oxygen and water. The same enzyme is found in human blood.



5. **“Enzymes (catalysts in general) speed up a reaction without entering into the reaction.”**

Although a catalyst, or an enzyme, is the same after a reaction has occurred and appears to be unchanged, it plays a very active part in the reaction and undergoes changes that involves breaking and forming new bonds in the enzyme as well as in the substrate. Just as with other catalysts, the enzyme forms an *enzyme-substrate complex*, equivalent to the activated complex formed by most chemical catalysts.

6. **“Enzymes increase the amount of product formed by changing the equilibrium point of a reaction.”**

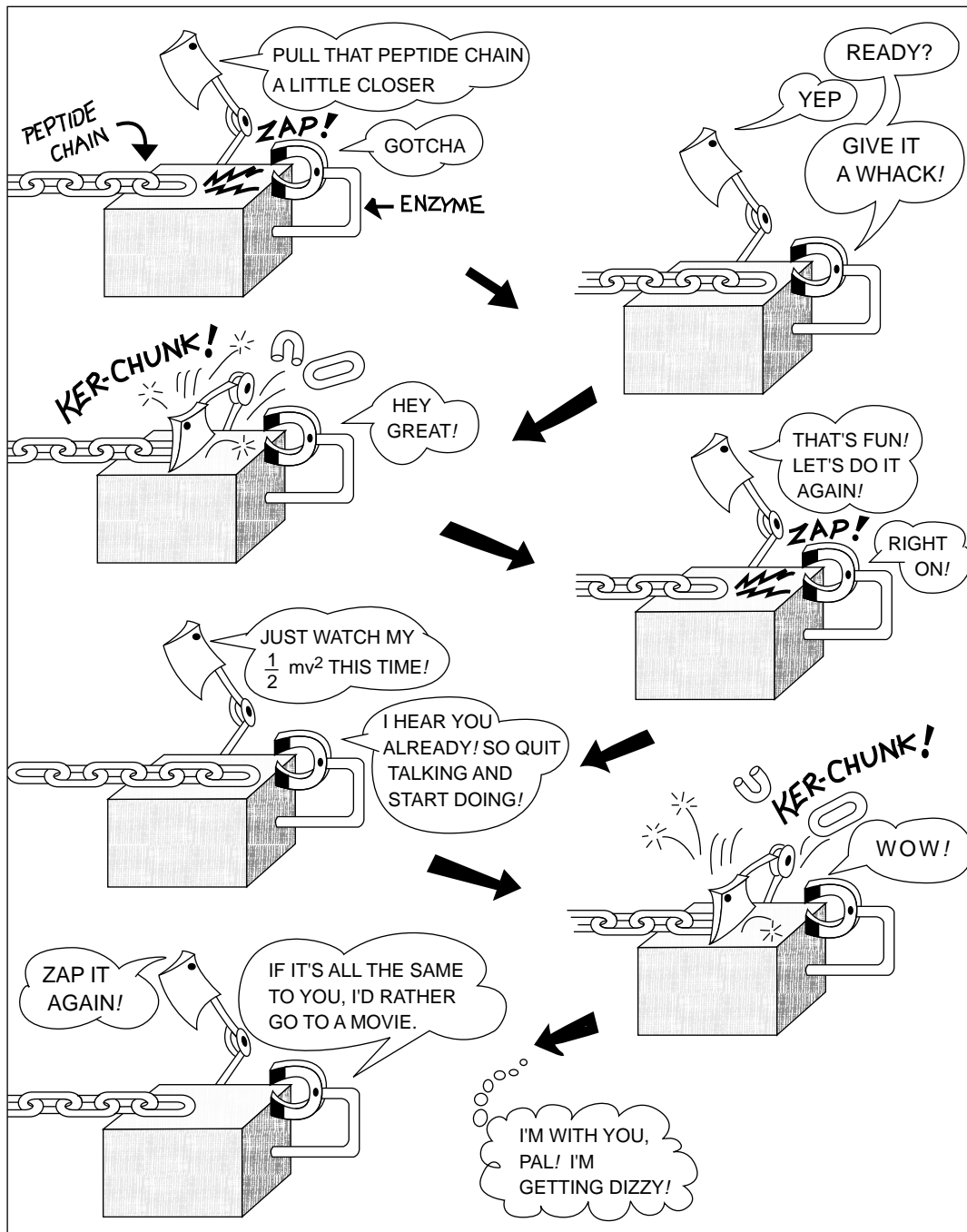
Enzymes do *not* change the equilibrium point of a chemical reaction. They only affect the rate at which the equilibrium point is approached.

HISTORY: ON THE HUMAN SIDE

- 1831** Berzelius assumed that certain substances contain a “*catalytic force*” that permitted them to accelerate a reaction.
- 1833** Jean Persoz and Anselme Payer prepared a malt extract that converted starch to dextrins. They called this substance *diastase* from the Greek word that means “breaking.” We now call this enzyme amylase.
- 1836** Theodor Schwann showed that a living cell (yeast) was required for fermentation.
- 1845** Mialhe discovered diastase in saliva and called it *animal diastase*.
Bouchardat, Sandras and Valentin found the same substance in pancreatic juice and suggested that the pancreas be called the *abdominal salivary gland*.
- 1876** Kuhne suggested the name enzyme, from the Greek word meaning *leaven*. Such substances were previously called “ferments.”
- 1883** As more enzymes were discovered, Duclaux suggested that all enzymes be named to end in *-ase*, as in *diastase*.
- 1897** Eduard Buchner accidentally isolated *zymase* from yeast and showed it to be the active enzyme in yeast.
Bertrand proposed the first enzyme-activation scheme: calcium and pectase to form the active enzyme, pectinase.
- 1913** Michaelis performed the first kinetic studies on enzymes.
- 1926** James Sumner crystallized *urease* from jack bean meal and showed that it was a protein.
- 1930** John Northrop crystallized *pepsin* and showed that it was also a protein. He suggested that *all* enzymes are protein.

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HUMOR: ON THE FUN SIDE





2. There's an interesting biochem caper—
A future world food supply shaper.
Seem's bacteria'll toil
To make protein from oil
Or just think—juicy steak from newspaper!
CHEM 13 NEWS, April 1975, p. 894
3. Word Search (see *Appendix* for master copy)

J E Z Y M O G E N O F P K A I U C
L D J D W H M B R H C P L I S H K
Y E I H E D I T P E P P A O O T C
X N K P W G G J T Q H O A P E R E
S A O R M P T V X A W L F D L N L
F T C I R E T O H P M A I S E Z Q
V U W X U K M E K M G F Z N C A N
E R A O A J L U C Y L X Z Y T M W
O A Q J N I N J B U X Y V N R R P
K T J Y X E J R S T M X I Y I N Z
K I E K J S Y I F E H O S P C Y R
O O Z J O A B I C A O N I M A U K
C N S I S E R O H P O R T C E L E

Words about the concepts in this module can be obtained from the clues given. Find these words in the block of letters:

1. Model of protein structure in which intrachain hydrogen bonding holds polypeptide chains together in a coil. (2 words)
 2. Building block of proteins. (2 words)
 3. Having both acidic and basic character.
 4. The loss of form and function of a protein.
 5. Type of bond formed between sulfur atoms of polypeptide chains.
 6. Technique used for separating proteins based on their molar mass and charge.
 7. pH point at which an amino acid has no net charge.
 8. Another name for the amide bond joining two amino acids.
 9. Biocatalyst protein.
 10. Another name for a proenzyme or inactive form of an enzyme.
- Answers: 1. ALPHA HELIX 2. AMINO ACID 3. AMPHOTERIC
4. DENATURATION 5. DISULFIDE 6. ELECTROPHORESIS
7. ISOELECTRIC 8. PEPTIDE 9. ENZYME 10. ZYMOGEN
4. See cartoons at end of module.

MEDIA 1. *The World of Chemistry* videotape "Number 23: Proteins: Structure and Function," is a good review of proteins with emphasis on primary and secondary structure. World of Chemistry Videocassettes. Annenberg/CPB Project, P.O. Box 1922, Santa Barbara, CA 93116-1922; (800) 532-7637; World of Chemistry Series, Atlantic Video, 150 South

Gordon Street, Alexandria, VA 22304; (703) 823-2800 or QUEUE Educational Video, 338 Commerce Drive, Fairfield, CT 06430; (800) 232-2224.

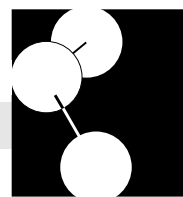
2. The following programs are available from Queue Educational Video, 338 Commerce Drive, Fairfield, CT 06430, (800) 232-2224:
 - a. *Digestive Systems*. JNS4048A Apple II's, 64K Disk (Emphasizes digestion. Allows student interaction.)
 - b. *The Basis of Biology: Biochemistry*. COM4232A, Apple II's, 64K, Disk and COM4233B, IBM PC, 128K, Disk. (Animated demonstrations of metabolism, including proteins, ATP, coenzymes, RNA, and DNA.)
 - c. *Chemistry of Life*, Apple II, IBM-PC, Macintosh. (Tutorial for extensive review of cell chemistry.)
 - d. *Enzyme Investigations* (HRM Software), HRM543A Apple II's. (Interactive introduction to enzymes and the digestive system.)
 - e. *Biochemistry Unit*. BI04012A, Apple II's. Five disks (Basic biochemistry.)
3. The following programs are available from Cambridge Development Laboratories, Inc., 214 Third Avenue, Waltham, MA 02154.
 - a. *Functional Chemistry in Living Cells* (HRM Film/Video) 7-HRC-054F. (A series of videos/films that explore the chemistry of life from the perspectives of cells and cell functions.)
 - b. *Proteins* Apple II's. 3-BLB-244A (Tutorial presentation of properties of proteins.)
4. Software published by Project SERAPHIM, Department of Chemistry, University of Wisconsin-Madison, 1101 University Avenue. Madison, WI 53706-1396: (608) 263-2837 (voice) or (608) 262-0381 (FAX).
 - a. For the Apple II computer: AP 726 (*Protein Structure Prediction and Restriction Map Simulator*)
 - b. For IBM PCs and PC-compatibles: PC 4901 (same as AP 726)
5. Videodisc published by *JCE: Software*, a publication of the *Journal of Chemical Education*, Department of Chemistry, University of Wisconsin-Madison, 1101 University Avenue. Madison, WI 53706-1396: (608) 262-5153 (voice) or (608) 262-0381 (FAX).

"The Boat and Dock Model," a chapter on *The World of Chemistry: Selected Demonstrations and Animations*: Disc II (double sided, 60 min.), Special Issue 4.

1. Vernier's Voltage Plotter with pH electrode. Price: approx \$100.
2. Nester model and Corning model pH meters (Flinn Scientific, Inc. Chemical Catalog/Reference Manual, 131 Flinn Street, P. O. Box 219, Batavia, IL 60510-0219). Price range \$25-700.
3. The following enzyme kits are available from Flinn Scientific Co., P.O. Box 219, 131 Flinn Street, Batavia, IL 60510.
 - a. *Enzymes: A Qualitative Approach* AP 1771 (Classroom quantities of materials needed to show how invertase and protease activity may be detected.)
 - b. *Biotechnology Enzymes in the Home* AP1770 (Kit of materials to show industrial applications of certain enzymes.)
4. The following instructional kits are available from Fisher Scientific Co., Educational Materials, Division, 4901 West LeMoyne Street, Chicago, IL 60651.
 - a. Chromatography/Electrophoresis Kit S40085ND (All apparatus and materials for 41 activities for separating materials by paper chromatography and electrophoresis.)
 - b. Electrophoresis Kit A14077. (Apparatus for separation of proteins and amino acids. Requires power supply unit sold separately [S40087ND].)

INSTRUMENTATION

Links/Connections



WITHIN CHEMISTRY

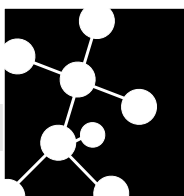
1. Chemical Kinetics
2. Acid/Base
3. Biochemistry (proteins)
4. Stereochemistry (isomers)

BETWEEN CHEMISTRY AND OTHER DISCIPLINES

1. **Nutrition.** As enzyme activators, vitamins and minerals are dietary essentials.
2. **Medicine.** Abnormalities often result if specific enzymes are missing. Over one hundred disorders due to deficient activity of a specific enzyme have been demonstrated in humans (see text by Holum in *References*).
3. **Pest Control.** Studies are under way to identify the enzyme that allows the cockroach to synthesize its exoskeleton. Since the roach can not live without this crusty shell, inactivating this enzyme will help eradicate this pest.

TO THE CONTEMPORARY WORLD

1. Fermentation results because of enzymatic action.
2. The enzyme *streptokinase* is often injected into the blood stream of patients who have suffered a heart attack from clots in the coronary arteries. The enzyme often dissolves the clots and saves the patient.
3. *Proteinase* is a general term for enzymes that helps dissolve spots and stains on clothing. They are used extensively in laundry detergents and are produced in larger quantities than any other commercial enzyme.
4. Many enzymes are used in food processing, including:
 - a. **Amylase:** Breaks down starch to produce more sugar for fermentation. Used extensively in bakery products, brewing, and production of syrups, sugars, and cereals.
 - b. **Catalase:** Used in the “cold pasteurization” of milk.
 - c. **Cellulase:** Helps break down cellulose in coffee beans.
 - d. **Invertase:** Converts sucrose to glucose and fructose for a sweeter taste. Used in honey and candies.
 - e. **Pectinase:** Used to break down cell structures in fruits, increasing the amount of juice (also applesauce from apples).
 - f. **Rennet:** Coagulates casein in milk, producing curd.
5. Sewage Treatment. Both amylase and proteases are used to break down insoluble carbohydrates and proteins in sewage.
6. Amylases are used in the paper industry as binding agents for starch and other fillers used to make paper. They are also used to desize fabrics in the textile industry.
7. Proteases are used to prepare leather by removing hair and other material from hides. They are also used in the photography industry to reclaim silver from used film and developer.



Extensions

1. Have students do library research on enzymes involved in digestion. For example, *amylase* catalyzes the breakdown of starch; *lipases* catalyze the breakdown of fats, *etc.*
2. Have students talk with physicians about *Inborn Errors of Metabolism*. Two common examples are phenylketonuria and galactosemia.
3. Many states have laws that require the screening for certain enzyme defects in the newborn. Have students visit state or local health departments to learn about their policies.
4. Many animals (horses, cows, goats, termites, *etc.*) have bacteria in their intestinal system that produce the enzyme *cellulase*. This enzyme breaks down cellulose and permits these animals to digest grass, wood, paper, *etc.* Unfortunately, humans do not have the bacterium that produces this enzyme. Have students discuss the theoretical ramification if this enzyme could somehow be made available to humans.
5. All vitamins (except A, D, E, K) serve as enzyme *activators* in biochemical reactions. Have students do library research to learn more about the specific role of each vitamin.
6. Try gelatin (Jello™) prepared with fresh pineapple and also with canned pineapple. Why does the preparation made with fresh pineapple not gel?
7. Have students prepare reports on the importance of enzymes in cooking or home products.

References



Module developed by Robert Sol Davis, Patricia Owens, and Lee Summerlin, the Alabama team.

Borgford, C. L., and Summerlin, L. R. (1988). *Chemical activities*. Washington, DC: American Chemical Society.

Ornstein, A. (September, 1993). Alcohol denatures proteins, *Chem 13 News*, p. 31.

Holum, J. R. (1990). *Fundamentals of general, organic, and biological chemistry* (4th Ed.). New York, NY: John Wiley & Sons.

Chapter 24 contains a good description of enzymes, how they work, and their usefulness in medicine.

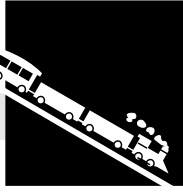
Novo Laboratories, Inc. (1975). *Enzymes, nature's catalysts*. Burlington, NC: Carolina Biological Supply.

Lehninger, A. L. (1988). *Biochemistry*. New York, NY: Worth.

Summerlin, L. R. (1981). *Chemistry for the life sciences*. New York, NY: Random House.

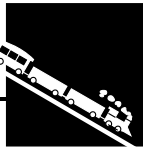
Summerlin, L. R., Borgford, C. L., and Ealy, J. B. (1988). *Chemical demonstrations: A sourcebook for teachers* (Vol. 2, 2nd Ed.). Washington, DC: American Chemical Society.

Summerlin, L. R. (1979). *Chemistry of common substances*. Morristown, NJ: Silver-Burdett.



Appendix

- **Transparency Master**
Word Search
- **Humor**



Word Search

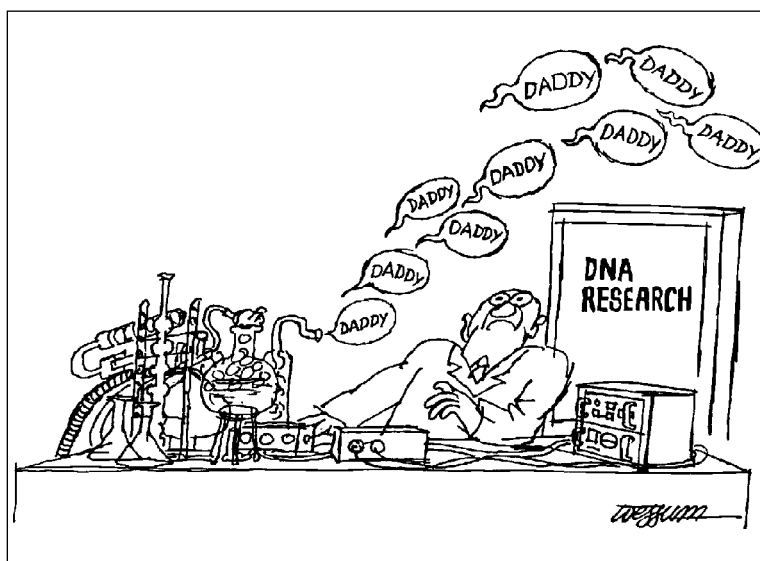
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L D J D W H M B R H C P L I S H K
Y E I H E D I T P E P P A O O T C
X N K P W G G J T Q H O A P E R E E
S A O R M P T V X A W L F D L N L
F T C I R E T O H P M A I S E Z Q
V U W X U K M E K M G F Z N C A N
E R A O A J L U C Y L X Z Y T M W
O A Q J N I N J B U X Y V N R R P
K T J Y X E J R S T M X I Y I N Z
K I E K J S Y I F E H O S P C Y R
O O Z J O A D I C A O N I M A U K
C N S I S E R O H P O R T C E L E

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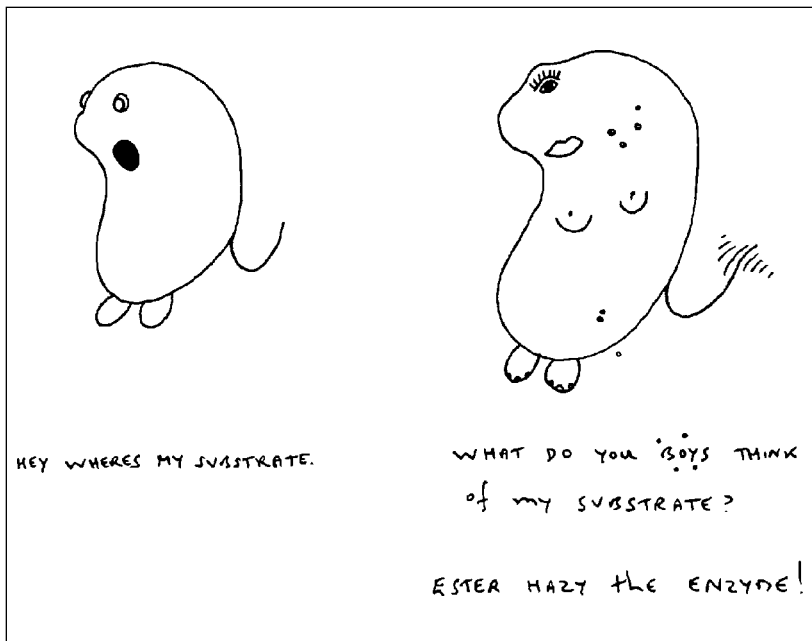
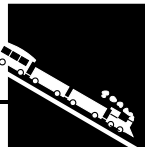
1. Model of protein structure in which intrachain hydrogen bonding holds polypeptide chains together in a coil. (2 words)
2. Building block of proteins. (2 words)
3. Having both acidic and basic character.
4. The loss of form and function of a protein.
5. Type of bond formed between sulfur atoms of polypeptide chains.
6. Technique used for separating proteins based on their molar mass and charge.
7. pH point at which an amino acid has no net charge.
8. Another name for the amide bond joining two amino acids.
9. Biocatalyst protein.
10. Another name for a proenzyme or inactive form of an enzyme.



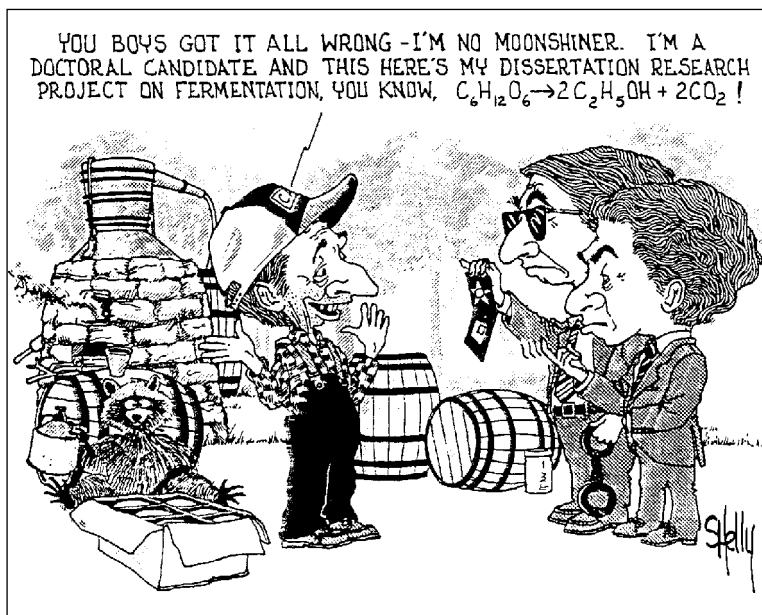
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