Dolan
DNA Learning Center
www.dnalc.org

Industrial Enzymology:
Cheese Production

Background

Most of the 100 trillion cells in the human body (except mature red blood cells and reproductive cells) contain the entire human genome--all the genetic information necessary to build a human being. This information is encoded in 3.2 billion base pairs, which are subunits of DNA. Inside the cell nucleus, 2 meters (about 6 feet) of DNA are packaged into 23 pairs of chromosomes (one chromosome in each pair is inherited from each parent). Human cells have 46 chromosomes that contain the DNA for approximately 30,000 individual genes (the units of heredity). Each gene is a segment of double-stranded DNA that holds the recipe (or code) for making a specific molecule, usually a protein.

In eukaryotes, DNA never leaves the nucleus. For the DNA instructions to reach the rest of the cell, an enzyme called RNA polymerase must make an RNA copy of a gene, called mRNA (messenger RNA). The mRNA copy leaves the nucleus and travels to a ribosome where the message is read. The ribosome then makes the protein coded for by that gene. To make a strand of RNA, the DNA first unzips, or pulls apart. RNA polymerase, an enzyme, builds an RNA strand complementary to one half of the unzipped DNA. For every C in the DNA strand the RNA polymerase inserts a G; for every G a C; for every T an A. For every A, RNA polymerase inserts a U, or Uracil. RNA does not contain Thymine. The ribosome “reads” the message 3 bases at a time. Each combination of bases letters is called a “codon.” For example, “UCG” is an mRNA codon. Codons code for amino acids, the building blocks of proteins. The UCG codon codes for the amino acid Serine (Ser). Transfer RNA (tRNA) molecules attach and deliver the proper amino acids to the ribosome to create a long chain. This chain of amino acids folds up to form a protein.

Proteins

There are four main types of proteins: structural proteins, transport proteins, messenger proteins and enzymes. Structural proteins provide our bodies with support. Examples include actin and myosin, which make up much of our muscle tissue, and keratin, which is found in both hair and fingernails. Transport proteins carry molecules throughout our bodies. One example includes hemoglobin, the protein in red blood cells that carries oxygen. Messenger proteins allow cells in different parts of the body to communicate. Some hormones, such as insulin and human growth hormone (HGH) are messenger proteins.

Enzymes act as the construction workers of the protein world. They can build molecules as well as break them apart by catalyzing (speeding up) chemical reactions. The molecule that a given enzyme breaks down is called its substrate. Like a lock and key, enzymes will only react with substrates that fit their shape correctly. Once the substrate is broken apart or digested, the enzyme is free to catalyze further chemical reactions. Unless the shape of the enzyme is altered, it can catalyze reactions indefinitely. The process that changes the shape of an enzyme is called denaturing. When an enzyme is denatured, it usually cannot alter its shape to function again. Some factors that can cause denaturing include changes in acid levels or pH and temperature fluctuations.

Human Digestive Enzymes

Enzymes are produced throughout the digestive system. Each digestive enzyme targets a specific substrate. For example, lipases target fats, amylases target starch, and proteases target proteins.

Most of the chemical digestion of food occurs in the small intestine by enzymes produced in the pancreas or in the intestine itself. **Lactase** enzyme is secreted in the lining of the small intestine. Individuals who are lactose intolerant have a lactase deficiency, and as a result, the dairy sugar lactose doesn't get digested. Normally, lactose is digested into its two constituent ingredients, glucose and galactose. When lactose isn't digested, it can cause abdominal discomfort and bloating, vomiting and or diarrhea. This enzyme deficiency can be treated through supplementation. Individuals who are lactose intolerant can take a pill containing the enzyme when they eat to aid in the digestion of the dairy sugar lactose. Individuals can also purchase lactose reduced dairy products, such as milk or ice cream. Harnessing the reusable nature of enzymes, with very small amounts of lactase large quantities of lactose free milk can be produced.

Surprisingly, it is quite normal for lactase levels to decrease with age. Lactose intolerance is actually an ancestral trait. As mammals, the natural production of this enzyme is only necessary in infancy when a baby nurses from its mother. After this stage, most mammals discontinue ingesting lactose, and the enzyme production drops and/or stops. Naturally, because humans are mammals, this happens to us too! In many of us though, lactase production continues beyond infancy thanks to our nomadic ancestors. When humans began to collect and drink milk from other animals, lactase production began to change.

Catalase is another example of a human digestive enzyme. It is produced in the liver. As blood is filtered through the organ, catalase digests toxic hydrogen peroxide that is present in low levels as a byproduct of cellular respiration. The products of this chemical reaction are water and oxygen. This reaction can easily be reproduced in the classroom, but with calf's liver instead of human liver! There is a visible reaction when liver is placed in hydrogen peroxide. Violent bubbling is a sign that gas is being released, and an increase in temperature is a direct indicator of an exothermic reaction taking place.

Enzymes in Industry

In cheese production, enzymes are used to separate the curds and whey in milk. Cheese is the solid portion of milk (curd) that has been separated from the liquid portion (whey). The cheese-making process was actually discovered by our nomadic ancestors. They would travel great distances, carrying liquids in the stomachs of dead animals. When milk is placed in a stomach the enzymes of the stomach cause the milk to curdle, thus producing clumps of curds or cheese. By combining the enzyme **emporase** with milk, this chemical reaction can easily be observed.

Enzymes are also used in the production of juice. In the flesh of an apple, apple juice is stored in tiny particles surrounded by walls of pectin. Pectin is a sugar molecule, and must be destroyed to release the juice trapped within. The enzyme **pectinase** is used to perform this chemical reaction in juice factories today. Years ago, apples would be pressed and crushed to extract their juices. Today, with the help of pectinase, juice factories are able to increase their yield.

Enzymes and Health

The absence of a single enzyme can have a drastic effect on an individual's health. For example, a single enzyme deficiency in white blood cells can lead to immune disorders such as SCIDS (Severe Combined Immunodeficiency Syndrome) also known as the "boy in the bubble" disease. Sadly, David Vetter, the young boy for whom the disease was nicknamed passed away following a bone marrow transplant at the age of 11. Since his passing, 2 young girls suffering from the same disorder have been successfully treated with gene therapy. Their white blood cells were genetically engineered and transfused back into their bodies, bringing with them a trait they didn't have before: the ability to fight infection!

Description of Activity

This one-hour laboratory provides children in grades 5-8 with a view into the workings of enzymatic reactions. Using the enzyme emporase, students will be turning milk into cheese! Upon experimentation and observation, students will understand how enzymes can be used to produce certain foods.

Learning Outcomes

Students will:

- discuss the relationship between gene, proteins and enzymes.
- observe an enzymatically catalyzed chemical reaction.
- define the terms chemical reaction and catalyze.
- compare experimental results with and without using enzymes.
- discuss what an experimental "control" is.
- understand that various factors can affect the rate of an enzymatic reaction

Assumptions of Prior Knowledge

Students should be familiar with the structure and function of DNA and understand the relationship between genes and proteins.

Misconceptions

Students often connect cheese to cows and goats, but do not understand the mechanisms behind its production. They tend to associate enzymes only with the digestion of food, but fail to understand the role enzymes play in the world around them.

Lesson

Materials and Equipment

1 half gallon of regular milk
1 quart of buttermilk
1 package of clear plastic disposable cups
40 popsicle sticks
40 5"x5" squares of cheesecloth (gauze)
20 droppers
1 bottle of Emporase enzyme (100 ml)
1 hot plate or water bath that can be set at 55˚C
1 thermometer

Before Class

The day before:

- Aliquot 1ml of enzyme for each team. Keep refrigerated.

The day of the lab:

- Mix 800 ml of milk with 200 ml of buttermilk (this makes 1 liter).
- Heat mixture to 55˚C
- Prepare set-ups for each team consisting of 4 plastic cups, 2 squares of cheesecloth, 2 popsicle sticks and 1 dropper.

During Class

- Review the relationship between DNA, RNA, and proteins . Discuss the connection between proteins and traits. There are several classes of proteins, for example:
  Structural: make up physical structures (keratin in hair and nails)
  Hormones: Insulin (controls metabolism of glucose)
  Enzymes: Amylase (digests starch)
  Not all enzymes are proteins, but most are.
  Mention that the names of most enzymes and in "ase".
- Enzymes are the workhorses of the cell, and catalyze chemical reactions.
- Define "catalyze" and "chemical reaction". To "catalyze" a chemical reaction is to speed up the process of the reaction. For example, if you eat one potato and leave another out on a table, which will be broken down first? Without enzymes, it could take months for the potato on the table to even begin to break down.
- A chemical reaction is when molecules are changed (chemical bonds are broken or built). In a chemical reaction there are reactants and products. The products are chemically different than the reactants.
- This principle can be demonstrated with a piece of paper. Ripping it or dissolving it in water can physically change the paper, but to change it chemically it would need to be burned, soaked in acid or maybe even eaten! This demonstration can be used in conjunction with any enzymatic lesson.
- Emporase is a synthetic enzyme that can break up sugar branches that are attached to casein particles in milk. The sugar branches separate these casein particles, making the milk creamy. Once these branches are broken off, the casein particles clump together forming curds. The liquid portion (whey) seperates out. Therefore making "curds and whey".

- A similar chemical reaction occurs in the human digestive system. There is an enzyme called rennet that is found in the stomachs of young mammals, especially milk-fed calves. Rennet like emporase, seperates curds from whey.
- There is a story that the cheese-making process was actually discovered by our nomadic ancestors. While traveling great distances they would transport liquids in the stomachs of dead animals. When milk was carried this way, and exposed to higher temperatures from the sun, the remaining enzymes in the stomach lining caused the milk to curdle. For our ancestors, they found that the whey was drinkable and the curds were edible! In this experiment, students will make cheese by adding the enzyme emporase to milk.
- Pass out the Milk to Cheese protocol and review the diagram showing the chemical reaction. Discuss that for the enzymatic reaction to occur, the appropriate temperature and pH need to be achieved.
- Explain that the solution will be heated to 55˚C. How will we achieve the right pH? Your stomach is very acidic. We need to make the solution more acid for the enzyme to work. This is why we use a milk/buttermilk mixture. The buttermilk has a lower pH (more acidic) than the milk and will bring the solution to the right level of acidity.
- Ask the students to read the protocol then demonstrate each step. Discuss what an experimental control is, and emphasize that one partner will be the "control" and the other will be the "experiment". Another option, if there is not enough buttermilk for each student, then the teacher can do one control at the front of the room for comparison.
- All the cheese could be collected and clumped at the front of the room to demonstrate the student's yield of cheese (optional).

Analysis and Discussion

Upon completion of the lab, discuss whether the results were successful or not. Ask the students what factors may have contributed to the small amountof clumping in the control. Why was the "control" necessary to understand the results of the experiment? Ask the students to discuss how genetics related to enzyme production.

Further Explorations

Group Presentations

Students can investigate alternative methods of making different types of cheeses on the Internet. Can cheese be made with other biological elements, such as bacteria or fungi? Are there other types of food that can be produced with the use of enzymes? Ask the students to present their findings to one another.

Discussion

Enzymes are very popular not only in food production, but in the world of scientific experimentation. Discuss some scientific applications of enzymes.

Note: This lab can be used in conjunction or followed up with the "From Apples to Apple Juice" lesson.

Enzymes in Molecular Biology

Restriction enzymes are used to cut the DNA molecule, and ligase enzymes are used to glue DNA fragments together. Using the *DNA Interactive* website, investigate the use of enzymes in recombinant DNA technology, genetic engineering and DNA fingerprinting.
Go to:
www.dnai.org < Manipulation < Techniques < Cutting and Pasting to learn more about restriction enzymes.
Go to:
www.dnai.org < Manipulation < Techniques < Amplifying to learn about amplifying DNA for techniques such as DNA fingerprinting.

Resources

Books:

Discovering Enzymes. Dressler, David, and Potter, Huntington. Scientific American Library, NY. 1991.
A great resource on the discovery, anatomy, physiology and evolution of enzymes.

DNA Science. Micklos, David A., Freyer, Greg A., and Crotty, David A. Cold Spring Harbor Press, NY. 2003.
A laboratory manual on the history of recombinant DNA technology.

Have a Nice DNA. Balkwill, Fran and Rolph, Mic. Cold Spring Harbor Laboratory Press, NY. 2002.
An elementary level introduction to basic cell processes such as DNA replication and protein production.

Internet Sites:

http://www.dnai.org/index.htm

DNA Interactive: a commemorative Internet site devoted to the discovery of the double helix.
A Dolan DNA Learning Center Internet site

http://www.ygyh.org/
Your Genes, Your Health: A multimedia guide to genetic disorders.
A Dolan DNA Learning Center Internet site

http://www.rcsb.org/pdb/
The Protein Data Bank, by the Research Collaboratory for Structural Bioinformatics.
This site contains a database of the molecular structures of proteins, including enzymes.

http://www.alphabet-soup.net/goose/curd.html
Another way to make cheese without using buttermilk..

http://www.historyforkids.org/learn/economy/cheese.htm
The history of cheese.

Correlations

New York State
NYS Standard 4: Science
The Living Environment

- Organisms inherit genetic information in a variety of ways that result in continuity of structure and function between parents and offspring.
- Organisms maintain a dynamic equilibrium that sustains life.

National
Content Standard C: Life Sciences
Structure and Function in Living Systems

- Living systems at all levels of organization demonstrate the complementary nature of structure and function. Important levels of organization for structure and functions include cells, organs, tissues, organ systems, whole organisms and ecosystems.
- All organisms are composed of cells—the fundamental unity of life. Most organisms are single cells; other organisms, including humans, are multi-cellular.
- Cells carry on the many functions needed to sustain life. They grow and divide, thereby producing more cells. This requires that they take in nutrients, which they use to provide energy for work that cells do and to make the materials that a cell or an organism needs.
- Specialized cells perform specialized functions in a multicellular organism. Groups of specialized cells cooperate to form a tissue, such as a muscle. Different tissues are in turn grouped together to form larger functional units, called organs. Each type of cell, tissue, and organ has a distinct structure and set of functions that serve the organism as whole.
- The human organism has systems for digestion, respiration, reproduction, circulation, excretion, movement, control and coordination and for protection from disease. These systems interact with each other.

Reproduction and Heredity

- Every organism requires a set of instructions for specifying traits. Heredity is the passage of these instructions from one generation to another.

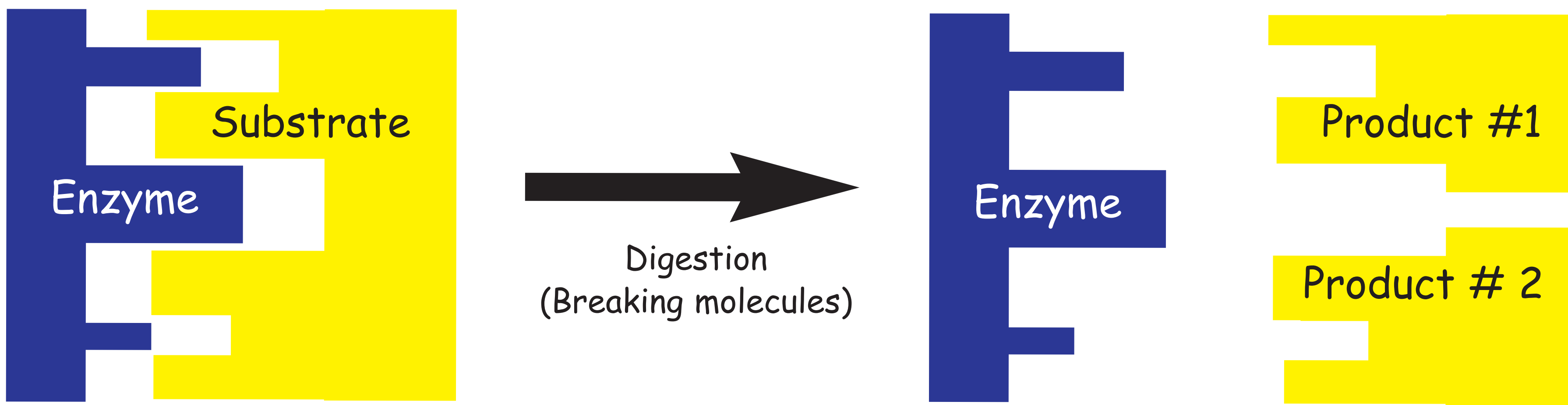
AAAS Benchmarks
Standard C: Cells

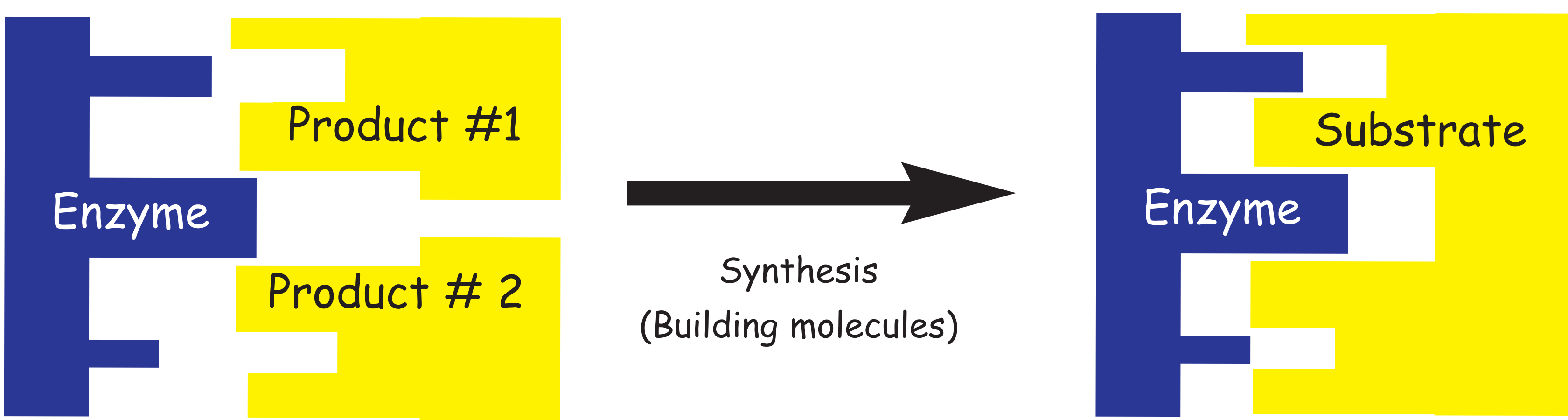
- Some living things consist of a single cell. Like familiar organisms, they need food, water and air; a way to dispose of waste; and an environment they can live in.
- Microscopes make it possible to see that living things are made mostly of cells.

Some organisms are made of a collection of similar cells that benefit from cooperating. Some organisms' cells vary greatly in appearance and perform very different roles in the organism.

Enzymes and Substrates

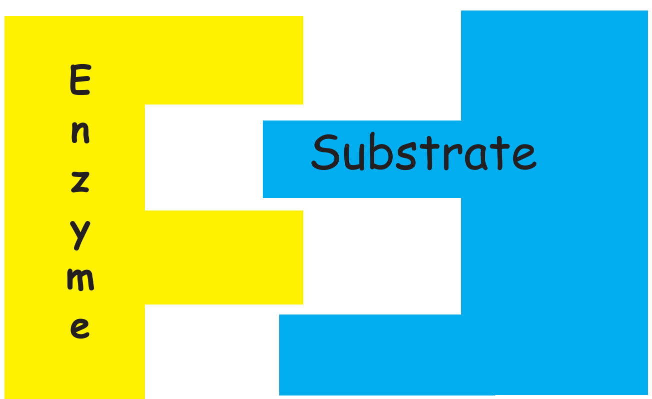
Enzymes, whether they break or build molecules, have specific substrates with which they can react. An enzyme and its substrate fit together like a lock and a key.



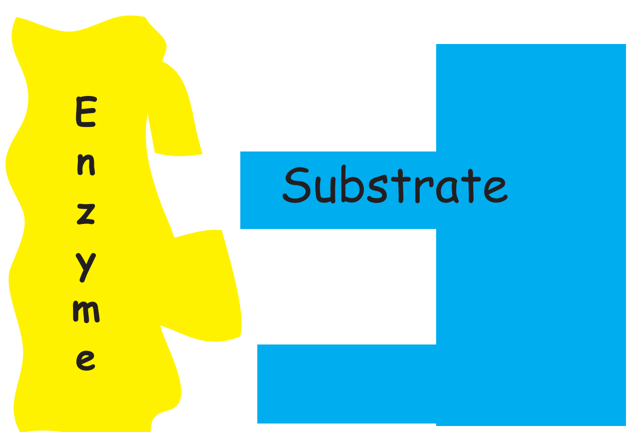


Enzymes and Substrates

If the shape of an enzyme is altered by a change in heat or pH, it can no longer bind with it's substrate. This change in shape is called denaturation. A denatured enzyme is like a melted key, it cannot fit with its substrate, and is non-functional.



The fit of an enzyme and its specific substrate.

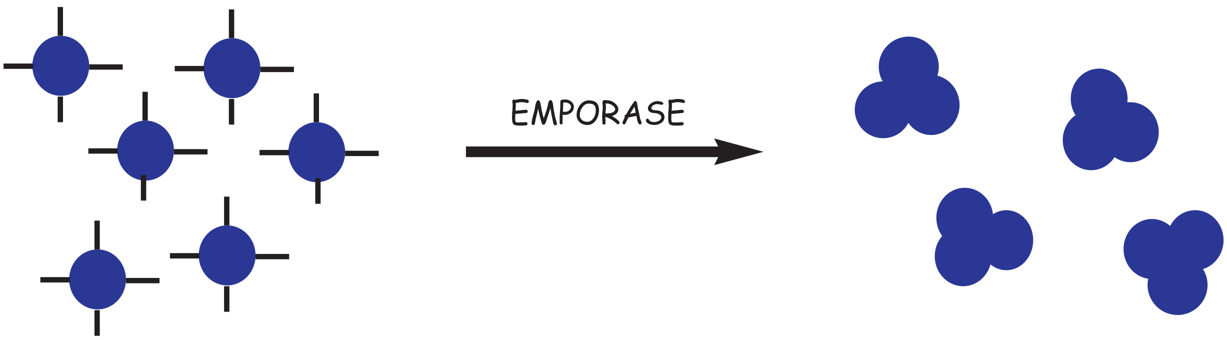


A denatured enzyme does not fit with it's substrate.

Milk to Cheese

Cheese is the solid portion of milk (curd) which has been separated from the liquid portion (whey). In cheese production, enzymes are used to separate the curds and whey in milk. The cheese-making process was actually discovered by our nomadic ancestors. They would travel great distances, carrying liquids in the stomachs of dead animals. When milk is placed in a stomach the enzymes of the stomach cause the milk to curdle, thus producing clumps of curds or cheese. In this experiment, we will combine the enzyme **emporase** with milk and observe this chemical reaction.

Emporase performs the following chemical reaction:



Casein particles (curds) are kept apart by small sugar branches in milk.

Casein particles clump together to form cheese when emporase breaks off the sugar branches.

Procedure:

1. Heat a solution of 100ml whole milk and 20ml of buttermilk to 55°C.

2. Label two plastic cups: **experiment** and **control**.

3. Fill each cup halfway with the milk/buttermilk solution.

4. Add 1ml of emporase enzyme to the experiment cup and stir.
   Allow both cups to sit for 5 minutes.

5. Label 2 clean plastic cups "experiment" and "control", and place a separate piece of cheesecloth over each.

6. While holding cheesecloth in place, pour both the experiment and control solutions into their respective cups. The curds should remain on top of the cloth, while the whey filters through into the cup. Compare the experiment and control.