

GK12 Module Teacher's Guide

Catalase
Don Benn

Abstract:

In this activity, students will determine the rate of reaction of the enzyme catalase. After knowing what a rate of reaction is, the students will then learn that a catalyzed reaction has products—water and oxygen.

Grade Level(s): 7th-8th

Objectives:

- Make critical observations using tables/graphs.
- Hypothesize outcomes based on their own dependent variables (e.g. enzyme/substrate concentrations).
- Perform a study about rates (non-mechanical) with biological approaches.
- Determine a rate of reaction of the enzyme catalase.

National Standards:

Standard A: Science as Inquiry; Abilities necessary to do scientific inquiry

Standard A: Science as Inquiry; Understandings about scientific inquiry

Standard B: Physical Science; Properties and changes of properties in matter

Standard C: Life Science; Structure and function in living systems

Standard C: Life Science; Regulation and behavior

Standard F: Science in Personal and Social Perspectives; Personal health

New Mexico Standards:

Strand 1, Standard 1: Scientific Thinking and Practice; Use scientific method

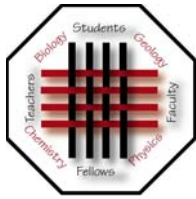
Strand 1, Standard 1: Scientific Thinking and Practice; Understand process of scientific investigation

Strand 1, Standard 1: Scientific Thinking and Practice; Use mathematical ideas, tools, techniques

Strand 2, Standard 1: Physical Science; Forms and properties of matter

Strand 2, Standard 2: Life Science; Structure and function of living things

Strand 2, Standard 2: Life Science; Structure of organisms, function of cells



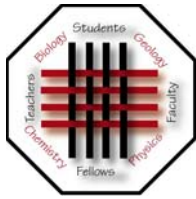
Materials:

- empty plastic soda bottles (2 liters)
- yeast extract (*Saccharomyces cerevisiae*, commercial packages)
- hydrogen peroxide (H₂O₂, commercial)
- water
- timer

Background:

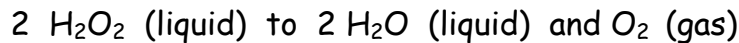
Protein composition and function is critical for cells to survive. This protein composition is derived from deoxyribonucleic acid (DNA). DNA, found in the nucleus of cells, is known to transmit heredity information from parents to their children. It is composed of four smaller components (nucleotides abbreviated as A, G, C and T) that are assembled in a very organized fashion. DNA eventually gets converted to protein in a very systematic process. Interestingly, DNA found in the nucleotide form has no specific cellular function (1). Proteins, on the other hand, are known to have very specific functions in cells.

In general, proteins are also made up of smaller components (amino acids) that, when assembled, produce much larger products (i.e. enzymes) that have cellular functions. Proteins are actually produced from nucleotides in the DNA that are organized into groups of three. The order of the nucleotides in the triplet codes for specific amino acids. These triplets are called codons. A codon is composed of three successive nucleotides and specifies which one of the 20 different kinds of amino acids will be used at a particular location in a protein (2). Each codon codes for either one of 20 amino acids, a START signal or one of three STOP signals. The same codons are assigned to the same amino acids and to the same START and STOP signals in the vast majority of genes in animals, plants, and microorganisms (3). One of the amino acids that can be found in proteins, phenylalanine (Phe, or W), for example, is coded for by either of two triplets (TTT and TTC). A host of other triplets can be determined by using a table known as the "Genetic Code" (Figure 1). (Note: There is another step in protein assembly that involves RNA, but for simplicity it will not be addressed). The common occurring amino acids are of 20 different kinds have in common an amino group (NH₂) and a carboxyl group (COOH)(Figure 2). It is the amino and carboxyl group that connect several amino acids up to form a polypeptide chain.



So, for a protein to form, several thousands to millions of these amino acids join to produce what is known as a polypeptide chain. A polypeptide chain is a natural or synthetic amide linkage whose formation occurs by the splitting out of water (2). The link is called a polypeptide bond, and it is shown on Figure 2 (4). The amino acids all have properties that give them specific characteristics. One example is chymotrypsin, which participates in digestion by breaking down some of the components in the food we eat. The active site of the protein chymotrypsin contains both a histidine (position 57) and an aspartate (position 102). If there is a missing aspartate at position 102, then chymotrypsin is inactivated and will not function. Ultimately, amino acids that are missing, occurring in the wrong sequence, or altered will affect protein function.

Proteins play very critical roles in cells. For example, the protein catalase converts hydrogen peroxide to water and oxygen in cells, as follows:



		2nd POSITION									
		T		C		A		G			
1st P O S I T I O N	T	TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys	T	3rd P O S I T I O N
		TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys	C	
		TTA	Leu	TCA	Ser	TAA	STOP	TGA	STOP	A	
		TTG	Leu	TCG	Ser	TAG	STOP	TGG	Trp	G	
	C	CTT	Leu	CCT	Pro	CAT	His	CGT	Arg	T	
		CTC	Leu	CCC	Pro	CAC	His	CGC	Arg	C	
		CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg	A	
		CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg	G	
	A	ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser	T	
		ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser	C	
		ATA	Ile	ACA	Thr	AAA	Lys	AGA	Arg	A	
		ATG	Met*	ACG	Thr	AAG	Lys	AGG	Arg	G	
G	GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly	T		
	GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly	C		
	GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly	A		
	GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly	G		

Figure 1: The Genetic Code

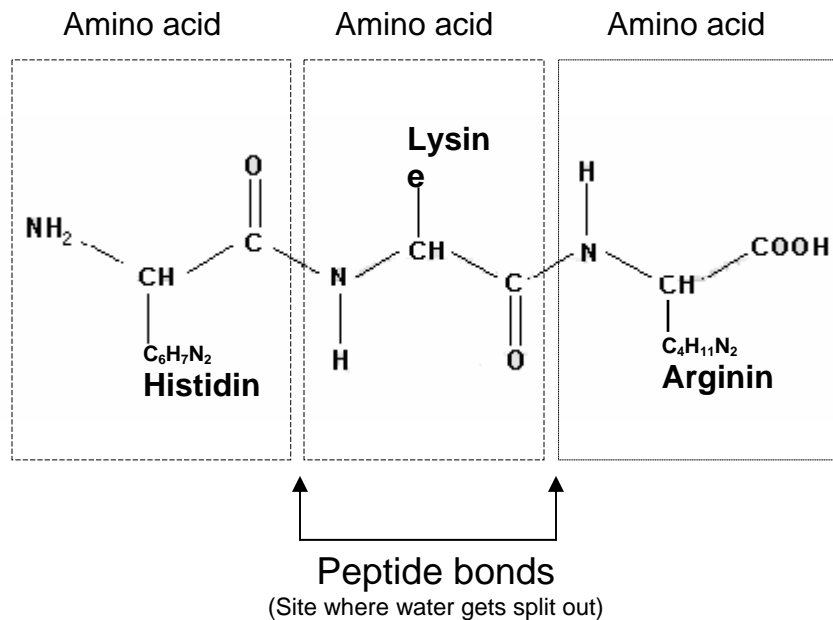
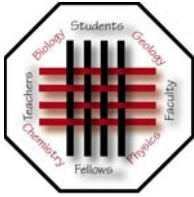
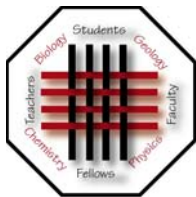


Figure 2: Peptide chain that contains 3 amino acids (histidine, lysine, arginine).

Why is catalase needed? It gets rid of peroxides that are harmful by-products of many cellular reactions. Catalase is found in the peroxisomes of cells. It has 4 polypeptide chains with each chain containing 500 amino acids each (approximately 2000 amino acids). Catalase breaks down and detoxifies hydrogen peroxide at a very high rate; one of the highest rates of all known enzymes: 40,000,000 molecules peroxide per second (5). If there is a single change in the protein sequence of catalase, such as in its active site, then it cannot break down hydrogen peroxide. Cells, in this case, would not exist due to the toxic nature of hydrogen peroxide, so it is crucial that catalase be intact to be active. There are a lot of other factors that affect the activity of catalase, such as temperature and pH.

Temperature affects the activity of most enzymes by speeding up a reaction, although high temperatures can also change or damage the molecular structure of proteins, thus inactivating it. A pH reading is a measure of how many hydrogen ions are in a solution. The scale can be confusing; lower numbers mean more hydrogen ions whereas, a higher number means fewer. A change in the pH environment of an enzyme causes it to either exist in an acidic solution (more hydrogen ions; pH 1.0-5.0) or basic solution (less hydrogen ions; pH 9.0-14.0).



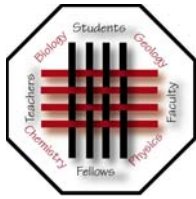
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In both cases, the changes produced in the chemical bonds of the enzyme molecule result in a change in shape that decreases enzyme activity (6). The majority of enzymes, like catalase, exist fine in solutions that are neutral (solutions that are pH 7.0). As long as catalase remains at 37 degrees Celsius and at physiological pH (7.0), it will remain active.

Proteins, such as catalase, control important biochemical reactions in cells. DNA and protein sequences are organized very systematically for individual enzymes and will affect its activity if altered. Alteration of either the DNA or protein shape occurs when certain factors, like temperature and pH, are changed. Such an alteration can change the rate of reaction or inactivate the enzyme. In the case of catalase, very toxic conditions occur, and cells die as a result of peroxide build up because the catalase can no longer breakdown the peroxide. So, it is very important, for optimal reaction rate, that proteins remain at certain conditions to remain viable.

References:

1. Watson, J. D. (2002) in *DNA from the Beginning: An animated primer on the basics of DNA, genes and heredity*, Retrieved May 4, 2004, Web site <http://www.dnaftb.org>
2. Metzler, D. E. (2001) *Biochemistry. The Chemical Reactions of Living Cells*, 2nd Ed., 1, Harcourt/Academic Press.
3. Kimball, J. W. (2003, June 6) in *Kimball's Biology Pages*, Retrieved May 5, 2004, Web site <http://biology-pages.info>
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Procedures:

Suggested activities to do prior to primary module:

- Introduce students to this module by learning about DNA gel electrophoresis first.
- Post vocabulary (about DNA, cells, nucleotides, amino acids, proteins, etc.) randomly on the walls of the classroom about a week in advance. This way the students are exposed to seeing the words in class before this lesson is actually taught.
- Show the students a model of protein (catalase), and discuss the parts.

Quick Module: Kinesthetic Protein Building

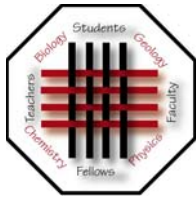
1. Have the students write down a letter (A,G,C or T) on a sheet of paper. Line the students up, at random, around the class room.
2. Have them use the "Genetic Code" to figure out which amino acid (triplets) three of them code for.
3. Tell the students which amino acids are either hydrophobic or hydrophilic. Tell the ones who make up hydrophobic amino acids to go to the center of the classroom.
4. This small activity will allow the students learn how proteins assemble themselves based on the properties of amino acids.

Primary Module: Rate of Reaction of the Enzyme Catalase

Question: What relevance does knowing about proteins have in their lives?

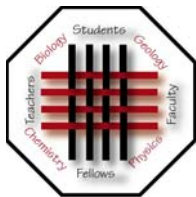
Remind the students of how DNA sequences are connected to protein production. Relate the module to Tay Sacs disease. Show this small movie (*One Wrong Letter*):

<http://www.pbs.org/wgbh/nova/genome/program.html#> .



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1. Cut the 2 liter plastic soda bottle about 8 cm. from the top. This will allow for a large mouth that the students can drop and retrieve the filter papers.
2. Fill the beakers/bottles up to an indicated line (approximately 1500 mL) with water.
3. Add 50 mL of hydrogen peroxide to the water. The teacher has to make the solution of hydrogen peroxide in advance.
4. "Pinch Out". This is a new term. It means that the students have to be the one who determines how much a "Large pinch" is to guesstimate what a medium and small pinch of yeast extract is.
5. Add:
 - "Small pinch" of yeast extract with 5 mL warm water
 - "Medium pinch" of yeast with 5 mL warm water
 - "Large pinch" of yeast with 5 mL warm water
6. Dissolve the yeast well.
7. Dip $(2.56\text{mm})^2$ filter papers in each yeast suspensions. Dap off excess water.
8. Drop the yeast paper into the beaker/bottle of dilute hydrogen peroxide. The sequences should be: "Small, Medium and Large pinch" samples.
9. Start time when it drops to the bottom of the containers. Stop timing when the paper floats and surfaces to the top.
10. Record time that it takes to FLOAT to the top.
11. Make a graph comparing time with amount of yeast added.



GK12 Module Glossary

Active site - Site or pocket on an enzyme that confines the substrate of a catalyzed reaction (i.e. catalase has a pocket where hydrogen peroxide sets).

Amide linkage - Bond between two amino acids, which consist of a carbon and nitrogen.

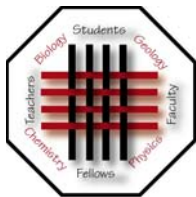
Catalyzed reaction - A reaction that increases the speed of a chemical reaction without changing the overall reaction process.

Catalase - An enzyme that speeds up the breakdown of hydrogen peroxide into oxygen and water.

Enzyme - A protein that speeds up the rate of chemical reactions without changing the chemical reaction.

Hydrogen peroxide - A toxic chemical that is produced naturally by organisms during their metabolism, but is immediately destroyed by an enzyme.

Substrate - Material or substance acted upon by an enzyme.



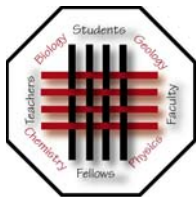
Catalase

This lab shows the rate of reaction of the enzyme catalase. Small pieces of filter paper are dipped into yeast suspensions (1-3) and dropped into a beaker of hydrogen peroxide. Then a table and graph of yeast suspension versus time will be done to show how fast the reaction took.

Procedure:

1. Fill beakers/bottles up to indicated line.
2. Add 50 mL of hydrogen peroxide to the water. (Ask the teacher to pour the hydrogen peroxide.)
3. Weigh out:

Small pinch of yeast	add 10 mL of WARM water
Medium pinch of yeast	add 10 mL of WARM water
Large pinch of yeast	add 10 mL of WARM water.
4. Dissolve the yeast well by swirling so that the yeast disappears.
5. Dip the small squares of filter paper in each yeast solution for 1 minute.
6. Shake off excess water.
7. Drop "YEAST PAPER" into the containers.
8. Start time when yeast paper drops to the **BOTTOM** of the beaker. Stop timing when the yeast paper surfaces at the **TOP**.
9. Record the time that it takes to float to the top.
10. Make a graph comparing time and amount of yeast pinches.



GK12 Module Student's Guide

Samples:	Time: minutes seconds
"Small pinch"	
"Medium pinch"	
"Large pinch"	

Questions:

1. When there is more yeast what happens to the reaction time?

2. What did the enzyme in the yeast do to the rate of reaction?