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Chemistry Comes Alive! Volume 5

Teacher's Guide and Student Activities for

HIV-1 Protease: An Enzyme at Work

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Teacher's Guide and Student Activities

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Foreword to Teachers

Please note that the video *HIV-1 Protease: An Enzyme at Work* is not designed to be a stand-alone instructional tool. Information and activities from this guide should supplement viewing and discussion of the video. The purpose of the entire package is to assist you in preparing lessons and discussions on the topic of HIV-1 protease, but the concepts taught are within the context of enzymology, protein chemistry, catalysis, and kinetics.

The graphics in the Teacher's Guide are designed to be photocopied onto overhead transparencies for easy presentation. (A full-page version of each figure is at the end of this document.) The guide assumes the reader has a working knowledge of basic chemistry but no prior knowledge of enzymology, protein chemistry, or virology is assumed.

The guide includes three student activities that use enzymes. Since students cannot perform experiments with HIV and HIV-1 protease for obvious safety reasons, the activities inp‰}de other examples of enzymes. An estimation of the time required and a brief activity summary are included in the Teacher's Notes for each activity.

Additional Information

For additional information on HIV-1 protease and AIDS research, please see:

Volker, E. J. An Attack on the AIDS Virus: Inhibition of the HIV-1 Protease; *J. Chem. Educ*. **1993,** *70*, 3–9.

Science **1993,** *260* (5112), 1253–1286. (Special issue on AIDs.)

Two articles with ideas for teaching about enzymes are:

Helser, T. L. A Dynamic Model to Demonstrate Enzyme Activity and Inhibition; *J. Chem. Educ.* **1991,** *68*, 286–287.

Berin, C. L. Enzymes: A Workshop for Secondary School Students; *J. Chem. Educ.* **1994,** *71*, 241.

Helser's article gives instructions for constructing a model of an enzyme and substrate from a foam football and Styrofoam balls. Berlin's article provides numerous ideas for conducting a workshop on enzymes.

Background Information on Proteins and HIV-1 Protease

Research for the treatment of persons infected with HIV has been underway for more than a decade. A potential treatment targets an enzyme known as HIV-1 protease. This protease is essential for the maturation of HIV. If an effective inhibitor, or drug, can be found to stop the protease from carrying out its functions in the virus, the replication of HIV could be stopped or the virus could be rendered non-infective. To oversimplify, the progress of the HIV infection might be kept under control by the natural immune system with the help of an inhibitor. Inhibition of HIV-1 protease could provide the key for the treatment of HIV and AIDS. Several drugs based on HIV protease inhibition are in the late stages of clinical trial and as of this writing (February 1996) one has been approved by the FDA for commercial sale. Researchers are working to find out more about how HIV-1 protease functions in the expectation of designing safer and more effective anti-HIV medication. A brief summary follows that describes the protease and its function.

Since the protease is an enzyme and, like most enzymes, a protein, your students will need a basic knowledge of proteins to discuss the protease. Proteins are long, folded chains of amino acids that are found in all living things. Later this guide will introduce the critical importance of protein folding and how it relates to biological function.

There are twenty common amino acids that make up proteins. All amino acids have a similar basic structure (Figure 1). The central carbon atom (the alpha carbon) bound to a hydrogen atom (H), an amino group ($–NH_2$), a carboxyl group (–COOH), and a side chain (R). In aqueous solutions at pH = 7 (approximately the pH of human body fluids) a carboxylic acid group (COOH) looses its proton to become a carboxyl anion ($–COO^–$). At the same pH an amine group ($–NH_2$) gains a proton to form an ammonium-ion-like protonated amine group ($–NH_3^+$). Thus the natural amino acids are zwitterions (dipolar ions having a cation-anion pair in the same molecule) at physiologic pH. If your students have studied acid-base equilibrium, this might be a good point at which to reintroduce these ideas.

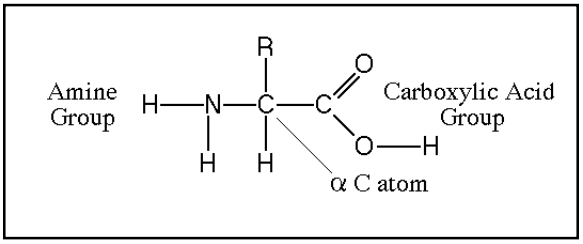


Figure 1a. Generalized structure of amino acid; different amino acids have different groups where the letter R appears on the α carbon atom.

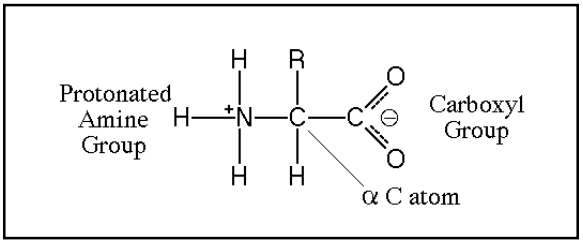


Figure 1b. Zwitterion structure of amino acid.

The composition of the side chain or group (R) attached to the alpha carbon is what makes one amino acid different from another. Among the common amino acids there are twenty different side chains, resulting in twenty different amino acids. Each amino acid is abbreviated by a three-letter code (Table 1). For example, the amino acid glycine is labeled *Gly* and the amino acid proline is abbreviated *Pro*. We mention proline because it is unique in that its amino group is also part of the side chain (a secondary amine) by way of a 5 membered ring. Proline is important to HIV-1 protease because the protease catalyzes breaking of a bond next to proline in a protein.

Amino acids are joined end to end by peptide bonds during protein synthesis (Figure 2). A reaction removes the elements of water and forms what is called a peptide bond between two amino acids. The process is repeated many times to lengthen the chain of amino acids. The amino group of the first amino acid and the carboxyl group of the last amino acid of a protein remain intact. The initial amino group is called the *amino terminus* and the final carboxyl group is called the *carboxyl terminus*. Formation of a succession of peptide bonds generates a backbone or main chain. The various side chains project from this backbone and determine how the protein will fold.

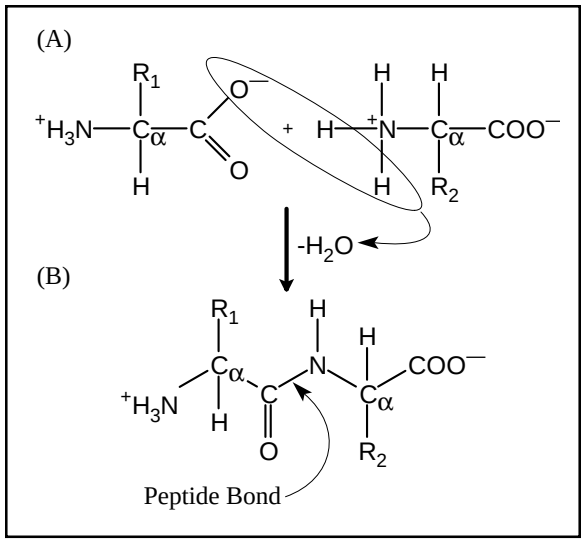


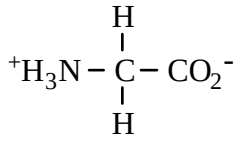
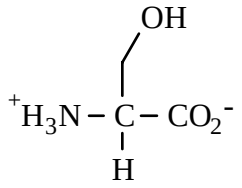
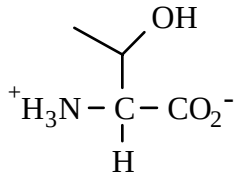
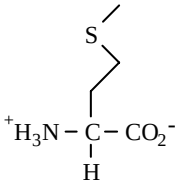
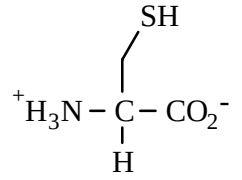
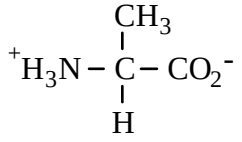
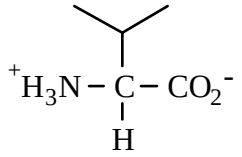
Figure 2. Peptide bond formed by eliminating water from amino acids.

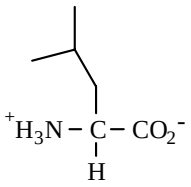
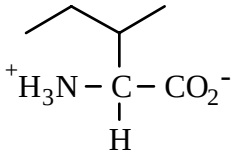
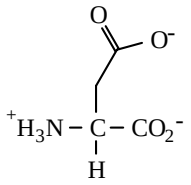
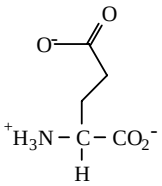
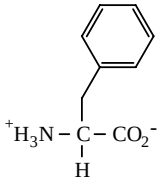
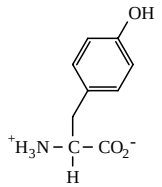
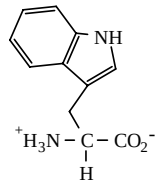
An amino acid sequence can be written using the three-letter abbreviations listed in Table 1. For example, the two amino acids joined by a peptide bond in Figure 2 could be glycine and alanine, where R_1 = H and R_2 = CH_3. The resulting dipeptide would then be written Gly–Ala and this nomenclature can be extended to any length peptide chain. An example is the amino acid sequence for HIV-1 given in Table 2.

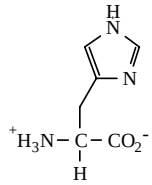
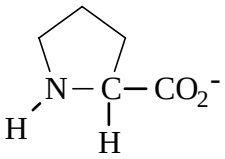
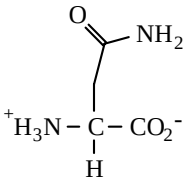
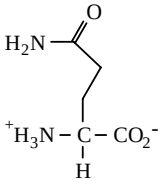
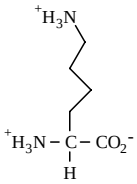
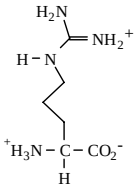
Another name for a long chain of amino acids is a polypeptide. The distinction between proteins and polypeptides is often based on origin and function. Usually the term *polypeptide* refers to portions of proteins or synthetically derived molecules, whereas *protein* is more specific and usually refers to molecules of biological origin with a complete structure for performing a biological function.

Table 1. The twenty common amino acids, three-letter codes, and structures.

To save space in these structures, carbon and hydrogen atoms are often not shown explicitly but rather indicated by ends of intersections of bond lines; for example, the R group in serine is $–CH_2–OH$. It is indicated by two lines and OH.

Side Chain (R)	Name	Three-letter code	Structure
H	Glycine	Gly	
Alcohol	Serine	Ser	
	Threonine	Thr	
Thiol, Disulfide	Methionine	Met	
	Cysteine	Cys	
Alkyl	Alanine	Ala	
	Valine	Val	

Side Chain (R)	Name	Three-letter code	Structure
Alkyl (continued)	Leucine	Leu	
	Isoleucine	Ile	
Carboxylic Acid	Aspartic Acid	Asp	
	Glutamic Acid	Glu	
Aromatic	Phenylalanine	Phe	
	Tyrosine	Tyr	
	Tryptophan	Trp	

Side Chain (R)	Name	Three-letter code	Structure
Special	Histidine	His	
	Proline	Pro	
Amide	Asparagine	Asn	
	Glutamine	Gln	
Base	Lysine	Lys	
	Arginine	Arg	

Each protein is defined by a unique sequence of amino acids encoded in an organism's genetic material. Protein chemists refer to the amino acid sequence as the primary structure of the protein. Different parts of the primary structure give rise to particular regions of defined molecular shape called the secondary structure.

Imagine a child's set of snap together beads where the beads are lopsided. If you put such beads together, the resulting chain is unlikely to be linear (straight). Further, if each kind of bead in the set is lopsided in a regular way, like the common amino acids, you might expect the bead chain to curve in a regular way such as a spiral. A spiral in a bead chain is analogous to protein secondary structure. You can also imagine that beads are free to rotate about the "bond" (the string) holding each bead in the chain, but that lopsided beads naturally fall into a certain orientation with respect to that bond. Likewise, amino acids have a preferred angular orientation along the backbone and the backbone folds as a result. This folding gives each protein a characteristic shape that is important in determining the protein's function. Two important examples of secondary structure are a spiral called an α helix and a ribbon called a β strand (see Figures 4 and 5 and their accompanying text).

When a number of secondary structural elements gather into a compact arrangement, something called a tertiary structure arises. Large proteins may be even further organized by folding two or more polypeptides into what is called a quaternary structure. You might also think of protein structure in architectural terms. Houses have common elements like doors, windows, and walls (secondary structure) made up of common materials like wood, masonry, and metal (primary structure). However, each house is characterized by how the materials and elements are put together (tertiary structure) into endless possibilities. If several housing units are gathered together you have an apartment or condominium (quaternary structure).

You might ask, "Why all the emphasis on folding and structure?" The answer to your question is that the structures of proteins are responsible for the functions that those proteins carry out. In some cases the structure of the protein as a whole is important to its function. This is true in membrane proteins. In other cases the structure is important because it holds certain molecules or parts of molecules (for example, amino acid side chains) in the correct spatial orientation to react chemically. This is true of an enzyme. The second case applies to the HIV protease enzyme because 97% of the protease's amino acid residues are devoted to holding the other 3% in the right environment to make and break bonds. Note that amino acids that participate in bond making and breaking are often not adjacent in the amino acid sequence but are held close together by the rest of the protein. If you change the structure by any means you will alter or eliminate the biological function of a protein. The next section should illustrate the importance of protein structure-function dependence.

Most enzymes are proteins that have evolved to function as catalysts for chemical reactions in living systems. Like all catalysts, enzymes increase the rate of a reaction by providing a reaction pathway with a lower activation energy than the uncatalyzed reaction path. Without an enzymatic catalyst, many biochemical reactions would not be completed within days or even years. Most enzymes are very specific and will catalyze only one reaction or a small number of closely related reactions. This enzyme specificity occurs because the reactant molecules must fit the enzyme for catalysis to occur, much as a key fits in a lock.

Enzymatic catalysis involves the enzyme and its substrate (which is what the reactant molecule is called). In some cases cofactors that modulate or assist the reaction are also required but in no case is the enzyme consumed. The enzyme has a particular region that attracts and guides the substrate to the enzyme in a process called docking. This attractive region is known as the active site and

it is also the site where the catalytic reaction will occur. When an appropriate substrate is bound to an enzyme, the substrate reaction site and enzyme active sites are brought into extremely close proximity (on the atomic scale) and the enzyme catalyzes the desired reaction. The transformed reactant molecule is then released from the active site with the enzyme unchanged. The enzyme is then ready to catalyze the desired reaction for the next reactant molecule.

Sometimes a molecule will bind tightly to an enzyme and fit in its active site but remain inert to the reaction the enzyme normally catalyses. These unreactive compounds are competitive enzyme inhibitors. When they fill the active site of the enzyme, they prevent the enzyme from catalyzing the reaction of the natural substrate. This process of competitive enzyme inhibition is the basis of the action of many drugs, including penicillin.

An enzyme can also be rendered inactive through denaturation. Any physical or chemical process that changes the structure of a protein and renders it inactive is a denaturation process. An example of this is the heating of an enzyme. The heating breaks hydrogen bonds and disulfide bonds in the enzyme and causes the protein to unfold. The enzyme cannot perform its function in the unfolded structure and the organism that is dependent on it is injured or killed. Clearly, heating up living tissue in hopes of denaturing a specific enzyme is not an effective means of fighting disease, but this kind of denaturation is the basis for fighting disease through cooking food.

Cleavage enzymes are a special class of enzymes. One role they fill is to cut apart protein precursors, or pre-proteins, to form biologically active proteins. An analogy can be drawn with plastic trash bags that come in a roll. All the bags are attached to each other, representing the pre-protein. The trash bags must be separated, or cleaved, from the roll in order to be used. The separated trash bags are the biologically active proteins. Cleavage enzymes appear to have evolved where rapid activation of a protein is needed. It takes less time to cleave an already-synthesized but inactive pre-protein in a few places to form active proteins than it would to make the large number of peptide bonds needed to synthesize active proteins directly from amino acids.

A good example of rapid activation capacity of cleavage enzymes is the clotting of blood. Clotting must occur very quickly to prevent excess blood loss. The enzyme thrombin shown in the video is a cleavage enzyme at the end of a cascade of other cleavage enzymes. The cascade of cleavage enzymes builds geometrically and forms clot tissue in seconds as compared to hours or days for other types of tissue growth. This clotting mechanism works because the blood plasma serves as a stockpile of fibrinogen, the substrate for thrombin.

HIV, the virus that causes AIDS, synthesizes many component proteins essential to its survival by joining amino acids into long chains called pre-proteins. But these pre-proteins aren't biologically active and must be cut apart to form biologically active proteins. HIV has a cleavage enzyme called HIV-1 protease that catalyzes the specific reactions for HIV to cut apart the pre-protein when the virus begins to replicate.

When HIV begins to replicate it first makes pre-proteins. Then these are cleaved to form the component proteins of a new virus particle. The protease-cleaved proteins are used in various parts of the new particle (Figure 3). One such protein

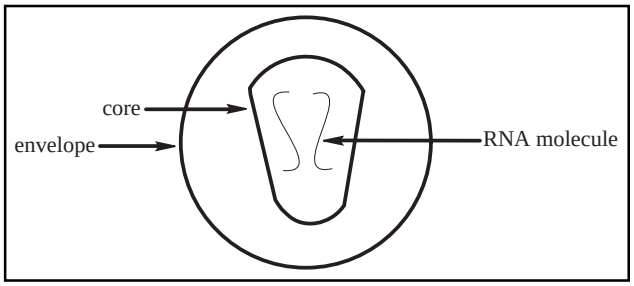


Figure 3. Schematic diagram of HIV.

is the matrix protein embedded in the virus's envelope. A second example is the capsid protein that forms the outer "skin" of the core of the virus. Inside the core of the virus particle, are two molecules of RNA. Each RNA molecule is tightly covered by a third protein, the nucleocapsid protein. All three of these example viral proteins (there are many others) are essential to the integrity of the new virus particle and all require HIV-1 protease to cleave a pre-protein before they become active. If you could inhibit the protease function, you would interfere with viral reproduction along several paths. This is why inhibiting the function of HIV-1 protease is such a worthwhile target.

A sequence of ninety-nine amino acids makes up the basic structure of HIV-1 protease (Table 2). Using three-letter abbreviations for the amino acids, proline, abbreviated Pro, is the first amino acid of the sequence. Phenylalanine, abbreviated Phe, is the ninety-ninth amino acid of the sequence.

Table 2. Amino acid sequence of HIV 1 protease.

Pro Gln Ile Thr Leu Trp Gln Arg Pro Leu Val Thr Ile Lys Ile [15]
Gly Gly Gln Leu Lys Glu Ala Leu Leu Asp Thr Gly Ala Asp Asp [30]
Thr Val Leu Glu Glu Met Ser Leu Pro Gly Arg Trp Lys Pro Lys [45]
Met Ile Gly Gly Ile Gly Gly Phe Ile Lys Val Arg Gln Tyr Asp [60]
Gln Ile Leu Ile Glu Ile Cys Gly His Lys Ala Ile Gly Thr Val [75]
Leu Val Gly Pro Thr Pro Val Asn Ile Ile Gly Arg Asn Leu Leu [90]
Thr Gln Ile Gly Cys Thr Leu Asn Phe [99]

The active protease has two identical protein molecules called monomers. The ninety-nine amino acid sequence is the primary structure of the protein and makes up one piece, or monomer of the protease (Figure 4). The monomer has secondary structures like β strands and an α helix and forms a complete tertiary structure. To form the complete protease, two of these monomers, join to form a dimer (Figure 5). The dimer is therefore a quaternary structure. Only the dimer functions as an active enzyme. Each of the protease monomers contributes half of the active site. The protease's active site consists of three amino acids from each monomer. This means that six amino acids make up the active site. They are the amino acids numbered 25, 26, and 27 in the sequence of 99. These three amino acids are aspartic acid, threonine, and glycine. As mentioned before, the active site is the place where the enzyme and the substrate come together and bind and it is also the place where the substrate is cut. In this case, the substrate is the pre-protein of the virus.

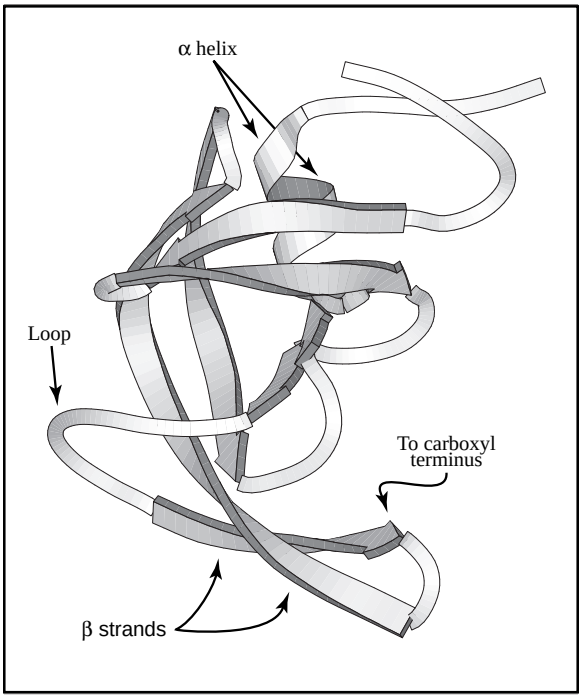


Figure 4. One monomer of HIV-1 protease.

The two monomers of the protease are attracted to each other and to the cleavage sites on the pre-protein. Intermolecular forces attract the monomers to form the dimer around the cleavage site. The active site of the protease matches up with the cleavage site of the pre-protein (Figure 6). The shape of the protease is similar to that of a doughnut, with the pre-protein extending through the doughnut hole. The monomers may also be attracted to each other to form a dimer around an inhibitor (Figure 7). The inhibitor stays bound to the protease. Since the inhibitor blocks the active site of the protease, the protease cannot perform its cutting function for HIV.

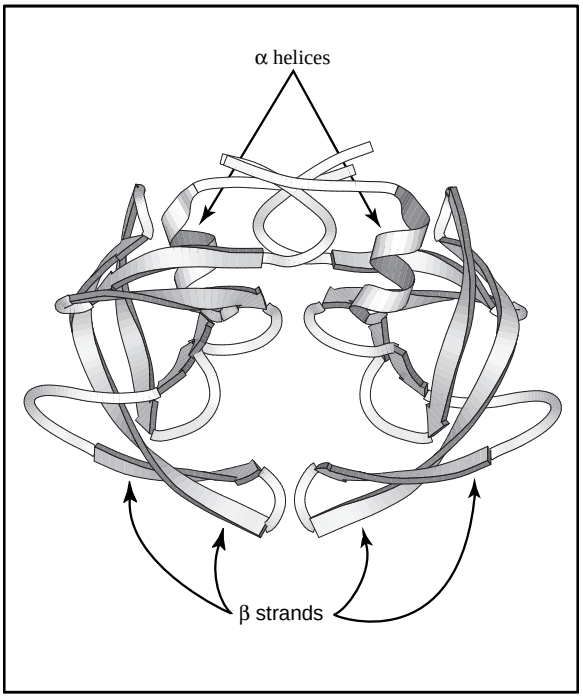


Figure 5. HIV-1 protease dimer.

Researchers are currently looking for safe and effective ways to inhibit HIV-1 protease and they have made some progress in producing drugs that prevent the protease from performing its enzyme functions. If the protease can be stopped, its inhibitor could be an effective drug for the treatment for HIV and AIDS. Researchers need to take many things into account when designing a drug to inhibit the protease. For example, the drug needs to affect primarily the protease, and not other enzymes of the human body, to minimize adverse effects to the patient. This is a problem in designing drugs to inhibit the protease because there are several other cleavage enzymes in the human body similar to HIV-1 protease. These include renin, which regulates blood pressure, and pepsin, which is a digestive enzyme.


Figure 6. HIV-1 protease dimer around pre-protein.

Unfortunately, not only is it difficult to design a safe and effective HIV protease inhibitor, but the HIV target is also constantly moving. HIV is genetically motile. This means that it rapidly adapts to changing environments by mutation. This rapid mutation means that even if a major breakthrough were to come today, tomorrow we could be back at square one. Clearly a point can be made for continuing research and deeper understanding.

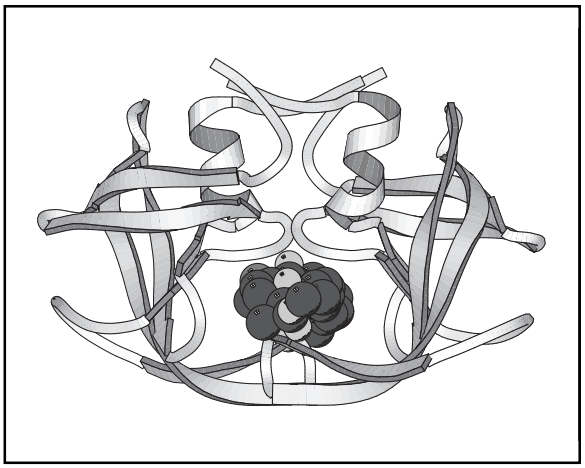


Figure 7. HIV-1 protease with inhibitor in the active site.

In Figures 4–7, you can see there are different ways to model a molecule like the protease. In Figure 4 and Figure 5, the protease is modeled as a combination of ribbons and coils. A coil model is like a tube or rope that runs through the center line of the protein backbone. The ribbons are flat and wider than the coils and they include the locations of all the backbone atoms. The ribbon model nicely illustrates that the atoms around the peptide bond are approximately planar. The ribbon pieces in the protease model each end in an arrowhead that points along the backbone toward the carboxyl terminus of each protease monomer. The ribbons and coils run through the protein's amino acid backbone. In the video, this mixed protease dimer model shows β strands as green ribbons, α helices as purple ribbons, and the remainder as gray coil. α helices and β strands are the two most common secondary structures (particular folding conformations) in a protein. In Figure 6, the protease is still modeled as a combination of ribbons and coils, while the pre-protein is modeled as a coil. In Figure 7, the protease model is the same, while the inhibitor is shown as a collection of spheres that are proportional to the size of the atoms they represent. The spheres illustrate how the inhibitor molecule fills up space in the active site.

Student Activity 1: HIV-1 Protease Inhibitor vs. Substrate

Researchers around the world have been searching for a treatment for HIV ever since the disease was discovered over a decade ago. Some researchers are working on a treatment that targets HIV-1 protease. The protease is an enzyme that helps HIV copy itself so it can infect other cells. Researchers are looking for molecules that can block the action of this enzyme. These molecules are called inhibitors. When designing molecules to inhibit the protease, researchers often look at the true substrate of the enzyme and design molecules similar to the true substrate. The protease's true substrate is a pre-protein shown in Figure 1.

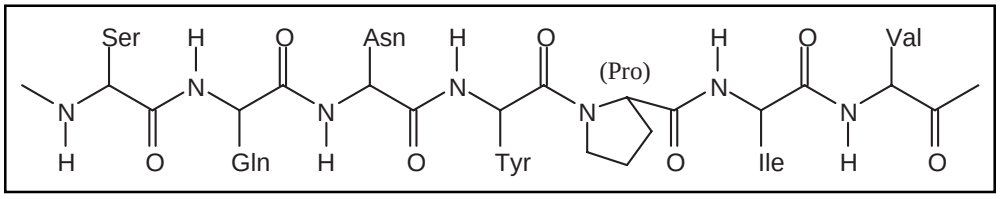


Figure 1. Fragment of pre-protein where it is cut by HIV-1 protease.

Researchers might change around some of the atoms, or substitute new ones to make a molecule to inhibit HIV-1 protease as shown in Figure 2.

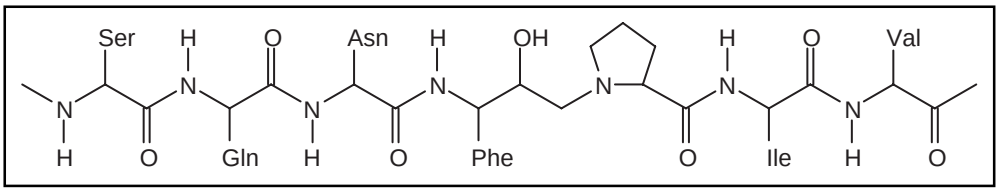


Figure 2. Central fragment of HIV-1 protease inhibitor, JG-365.

Questions

1. What are the differences between the pre-protein and the inhibitor?
2. Why do you think the inhibitor would work?
3. What does an inhibitor do to an enzyme?

Student Activity 2: One Potato, Two Potato

Potatoes are a source of the enzyme tyrosinase. The amino acid tyrosine can fit and bind to the active site of tyrosinase. When potato extract is combined with tyrosine and exposed to air, a series of reactions begins. The product of the reactions is a reddish-brown compound. In this experiment, you will investigate three other molecules (Figure 1) to see which ones act in a way similar to tyrosine based on molecular structure.

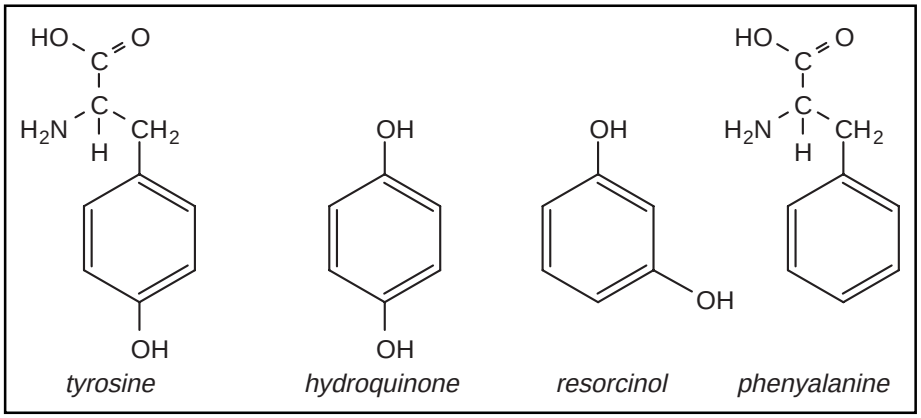


Figure 1. Molecules to investigate for reactivity catalyzed by tyrosinase.

Objectives

In this experiment you will:

1. Make a prediction based on molecular structure of which substance(s) will react in a way similar to the enzyme tyrosine to produce the reddish brown color.
2. Prepare potato extract to be treated.
3. Treat samples of the extract with a variety of substances and observe the results of the reactions over time.
4. Analyze results of data collected.
5. Apply information gained to another situation.

Equipment and Chemicals

mortar and pestle	10 ml graduated cylinder	distilled water
small beakers	5 clean test tubes and rack	cheesecloth
medicine dropper	water bath apparatus	thermometer

1% solutions of tyrosine, hydroquinone, resorcinol, and phenylalanine

0.10 M sodium fluoride solution

Procedure

1. Use the information provided and the molecular structures to predict which molecule(s) will act in a way similar to tyrosine. Explain your reasoning.
2. Prepare a table for your data to include test tube observations by letters A–E and time periods (initial, 5 min., 10 min., 15 min.).
3. Cut a piece of raw potato into small bits to yield about 10 ml total volume. Grind the bits into mush in a mortar and pestle. Add 10 ml of 0.10 M sodium fluoride solution to extract the enzyme. Mix well.
4. Strain the enzyme extract mixture through cheesecloth (or strainer) into a small beaker.
5. Label five clean test tubes with letters A through E.
6. Add the following to the labeled test tubes:

   A. 15 drops distilled water and 15 drops enzyme extract

   B. 15 drops 0.1% tyrosine and 15 drops enzyme extract

   C. 15 drops 0.1% hydroquinone and 15 drops enzyme extract

   D. 15 drops 0.1% resorcinol and 15 drops enzyme extract

   E. 15 drops 0.1% phenylalanine and 15 drops enzyme extract
7. Place all test tubes in a water bath at 37 °C and observe all tubes and record the initial color.
8. Observe all test tubes after 5 minutes. Record any color changes. Repeat this process after 10 minutes and after 15 minutes. Record all data in your table.

Questions

1. How do your results compare with your prediction? If your prediction was wrong, discuss how you now have to change your thinking about enzyme activity.
2. What purpose does test tube A serve in this experiment?
3. Tyrosine binds to the active site of tyrosinase with hydrogen bonds. What are hydrogen bonds? What part(s) of the tyrosine molecule is/are capable of hydrogen bonding?
4. Why was the experiment run at 37 °C? What might occur if the reaction was run at 10 °C?
5. If you had placed your test tubes into a bath of boiling water, you would not have observed a reddish-brown color in any of the tubes. Why?
6. From the molecular structure of catechol (Figure 2), could it bind to the active site of tyrosinase? Why or why not?

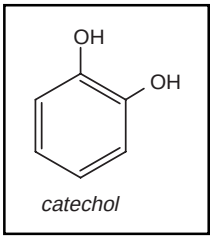


Figure 2. Catechol

Sample Data Table

Observations of substances in test tubes at various times

	initial	5 min.	10 min.	15 min.
Test Tube A (distilled water)				
Test Tube B (tyrosine)				
Test Tube C (hydroquinone)				
Test Tube D (resorcinol)				
Test Tube E (phenyalanine)				

Teacher's Notes for Student Activity 2

Estimated time table for activity:

10–15 minutes to discuss and make predictions (can be done the day before the activity)

50 minutes to perform activity and gather data

Time to write lab report and answer questions

In this activity, students see the molecular structure of tyrosine, which is the true substrate of the enzyme tyrosinase. They also examine the molecular structures of three other molecules, hydroquinone, resorcinol, and phenylalanine. Students then predict which of the three molecules will be able to fit into the active site of tyrosinase and react in a way similar to tyrosine. Students should make their prediction based on the molecular structures. Suggested questions for students are "Which structures are similar to tyrosine?" and "What effect would the groups on each of the molecules have on the ability of that molecule to fit in the active site of tyrosinase?" After students make their predictions, they test them. The activity helps students to view the similarity of a true substrate, tyrosine, to other molecules. They also see that a molecule similar to tyrosine can bond with tyrosinase and react in a manner similar to the tyrosine/tyrosinase reaction.

Answers to Questions

1. Experimental results show that the choice of hydroquinone is correct. A popular choice among students might be phenylalanine, since it has a structure very similar to tyrosine. However, phenylalanine doesn't have a hydroxyl group in the para position. The term para position means that the two groups on the ring structure are opposite of one another. This hydroxyl group is the deciding factor. The only other molecule that does have the hydroxyl group in the para position is hydroquinone, so it reacts similarly to tyrosine. The activity of enzymes often requires a particular group to be present in a particular position on the substrate.
2. Test tube A serves as a control. It shows whether the enzyme extract itself has any effects on a liquid (water) that does not contain one of the molecules such as tyrosine or hydroquinone.
3. Hydrogen bonds form between molecules or within a molecule. A hydrogen bond is the attraction between a slightly positive hydrogen atom on one functional group and a small, very electronegative atom, usually fluorine, oxygen, or nitrogen, on another functional group. The nitrogen and the three oxygen atoms on the tyrosine molecule can participate in hydrogen bonds.
4. The experiment was run at 37 °C, because most enzymes have a temperature at which they work best. The temperature at which tyrosinase works best is probably around 37 °C. Experiments could be done to find out the best temperature for tyrosinase activity. If the reaction was run at 10 °C, the enzyme would not function as well.
5. If the test tubes were in a bath of boiling water, the high temperature would denature the enzyme. This denaturing renders the enzyme ineffective. The reaction would not proceed, and the reddish-brown compound would not be produced.

6. Catechol might be able to react. Students at an introductory level of chemistry might say catechol probably would not be able to bond, because the hydroxyl group is not in the para position. After students have learned some organic chemistry, they might say that para and ortho groups on a molecule produce similar reactions. (The term ortho position means that the two groups are next to each other on the ring structure.) Since catechol has a hydroxyl group in the ortho position, it might bond to the active site of tyrosinase.

Acknowledgment

This experiment was adapted from Linda Kay Ford's *The Potato Experiment* published in the November 1987 issue of *The Science Teacher.*

Ford, L. K. *Science Teacher* **1987,** *54*, 44-45.

Student Activity 3: Gelatin and Protein Digestion

Gelatin is a protein found in connective tissues (ligaments and tendons). Enzymes such as papain, found in meat tenderizer, or bromelain, found in pineapple, denature the protein in the gelatin.

Objectives

In this experiment you will:

1. Prepare a gelatin solution.
2. Treat samples of the gelatin with a variety of substances and observe the results over time.
3. Analyze results of data collected; apply information gained to other situations.

Equipment and Chemicals

unflavored gelatin	meat tenderizer	water
four small plastic cups	fresh pineapple	
access to a microwave oven	canned pineapple	

Procedure

1. Make a 10% solution of unflavored gelatin and divide it into four equal portions in small plastic cups.
2. Allow gelatin to solidify. (The process can be hastened by placing the cups in the freezer or an ice bath.)
3. Generously pour meat tenderizer over the gelatin in the first cup. Let it sit for a while, possibly a few hours, and then test the consistency.
4. Place a few chunks of fresh pineapple in the second cup. Let it sit, then test the consistency.
5. Place a few chunks of canned pineapple into the third cup and observe what happens over time.
6. In the fourth cup, place some fresh pineapple that has been microwaved for a minute or two. Observe what happens over time.

Questions

1. For each treatment (meat tenderizer, fresh/canned/microwaved pineapple), how does the original consistency of the gelatin compare with its final consistency?
2. What does the canning process do to the pineapple?
3. Does microwaving the pineapple for a short time have the same effect as the canning process?
4. When making a gelatin dessert or salad containing pineapple, should you use fresh or canned pineapple? Why?

Teacher's Notes for Student Activity 3

Estimated time table for activity:

5–10 minutes for adding substances

30–40 minutes to observe changes

Time to write lab report and answer questions

In this activity, students observe what happens to gelatin samples when they are treated with different substances. The four substances used are meat tenderizer, fresh pineapple, canned pineapple, and fresh pineapple that has been microwaved. Meat tenderizer and pineapple each contain an enzyme that denatures the protein that makes up the gelatin. However, when the pineapple is either canned or microwaved, the enzyme in the pineapple is denatured. The denatured enzyme has no effect on the gelatin.

This activity can also be done as a teacher demonstration. One can then prepare the gelatin and/or add the substances before class.

Answers to Questions

1. Fresh pineapple and meat tenderizer denature the gelatin. However, the canned and microwaved pineapple have no effect on the gelatin.
2. The canning process denatures the enzyme in the pineapple.
3. Microwaving the pineapple for such a short time does not have the same effect as the canning process. If the pineapple was microwaved for a longer time, the enzyme would denature as it does in the canning process.
4. From the results of the lab, you should use canned pineapple. If you use fresh pineapple, the enzyme in the pineapple will denature the gelatin.

Acknowledgment

This experiment was adapted from *Molecules in Living Systems: A Biochemistry Module, Interdisciplinary Approaches to Chemistry* by David Martin and Joseph Savonpyra.

Martin, D.; Savonpyra, J. *Molecules in Living Systems: A Biochemistry Module, Interdisciplinary Approaches to Chemistry;* Harper & Row: New York, 1978.

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Overhead Transparency Masters

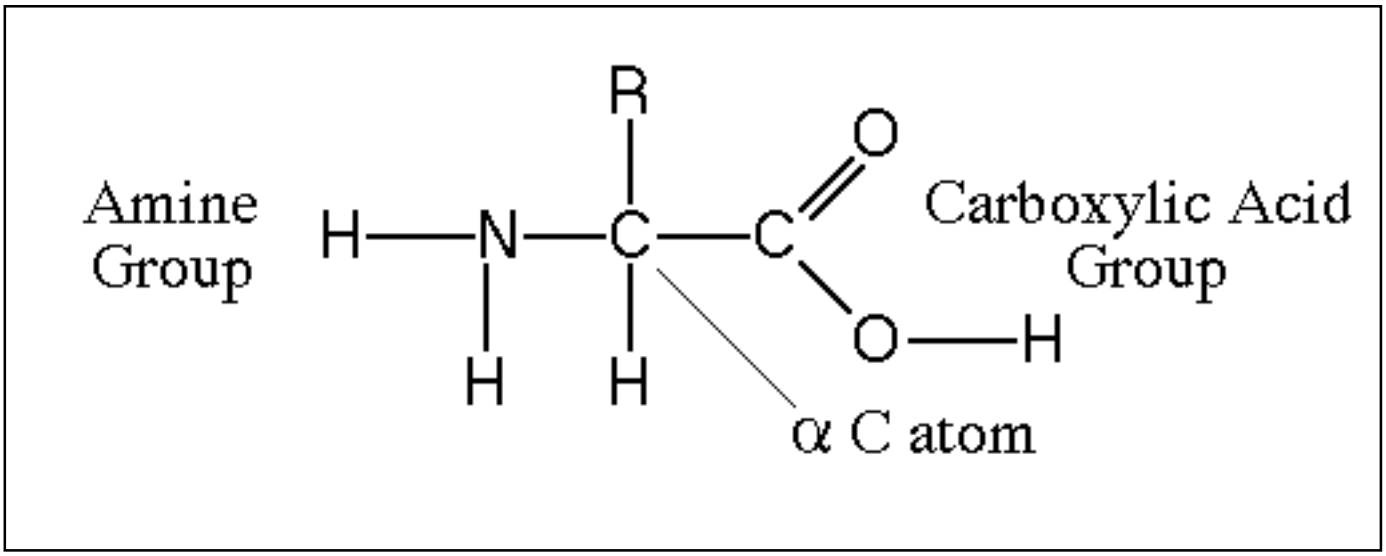

Figure 1a. Generalized structure of amino acid; different amino acids have different groups where the letter R appears on the α carbon atom.

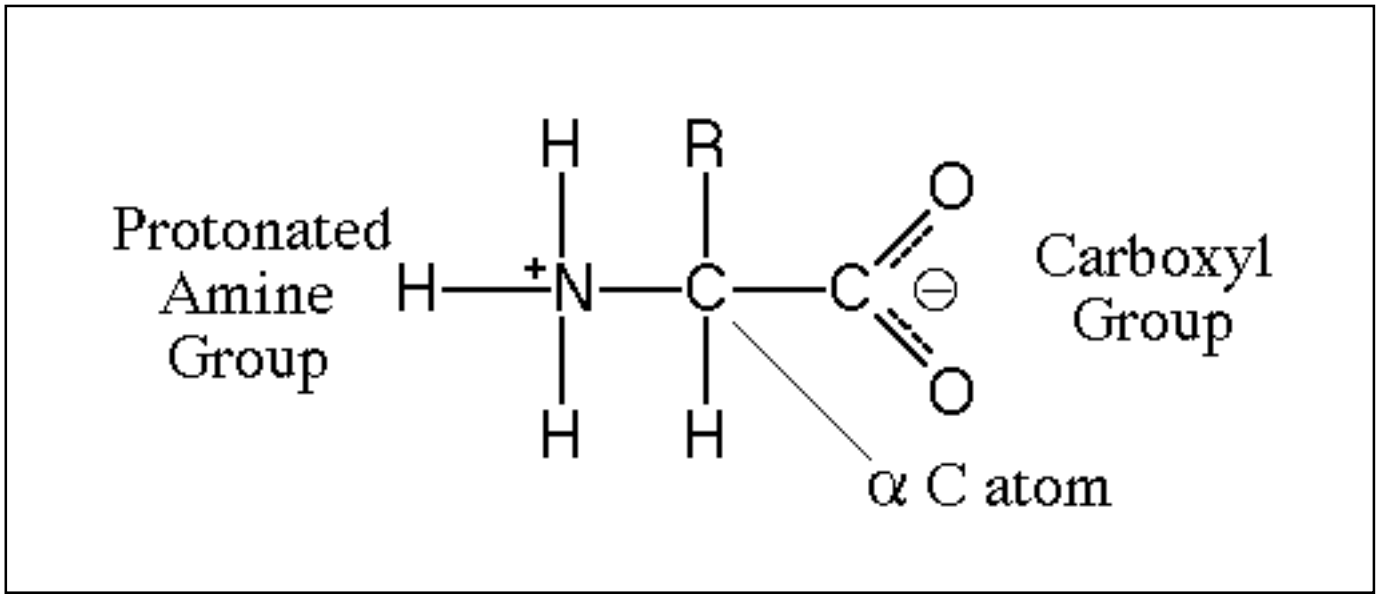

Figure 1b. Zwitterion structure of amino acid.

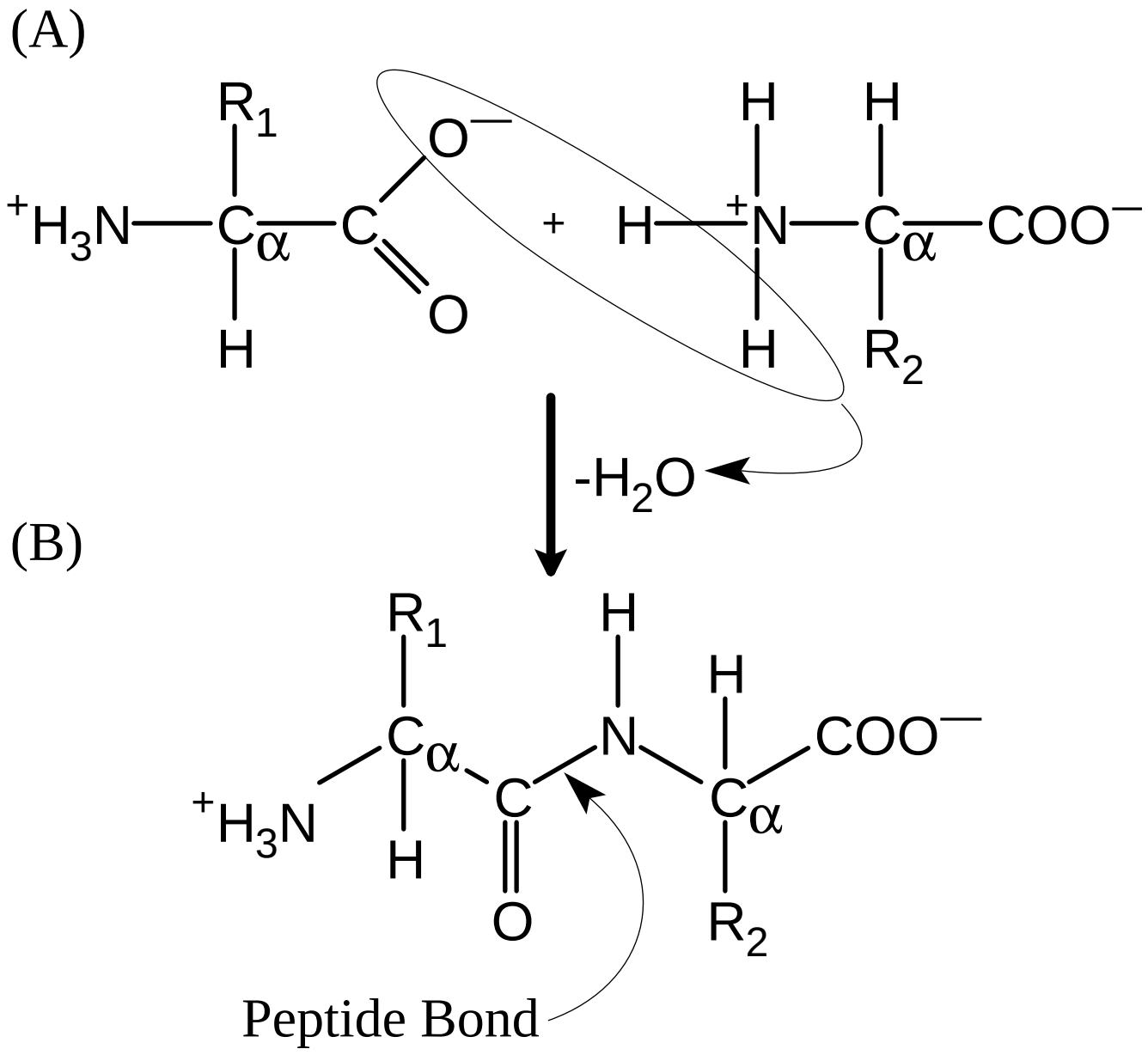

Figure 2. Peptide bond formed by eliminating water from amino acids.

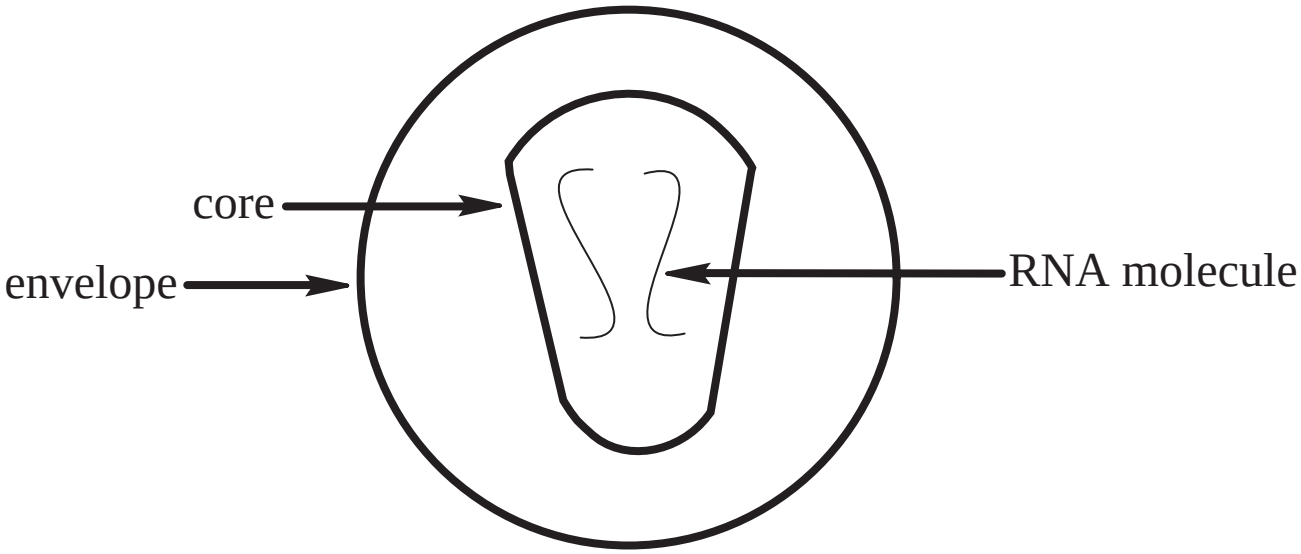

Figure 3. Schematic diagram of HIV.

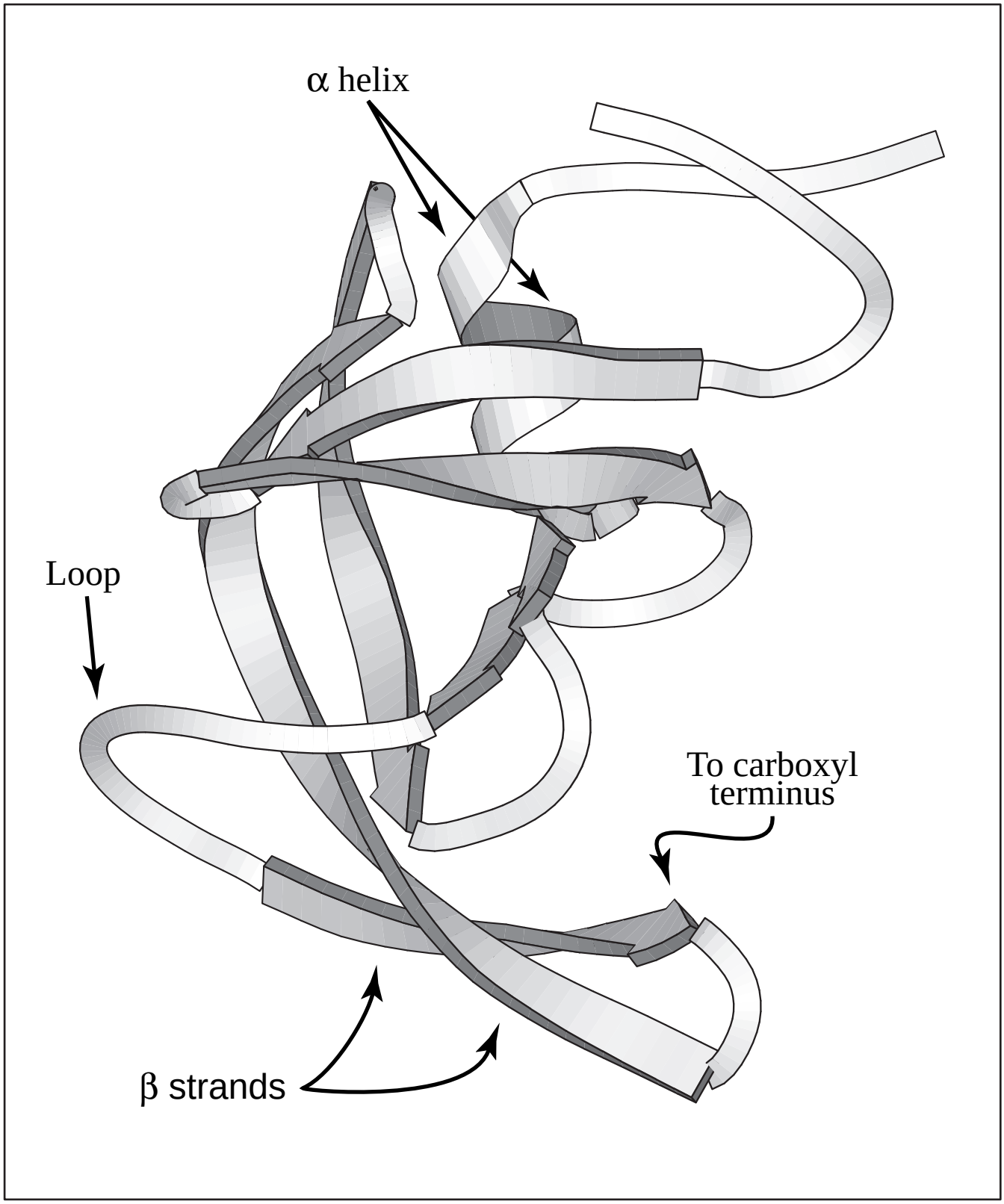

Figure 4. One monomer of HIV-1 protease.

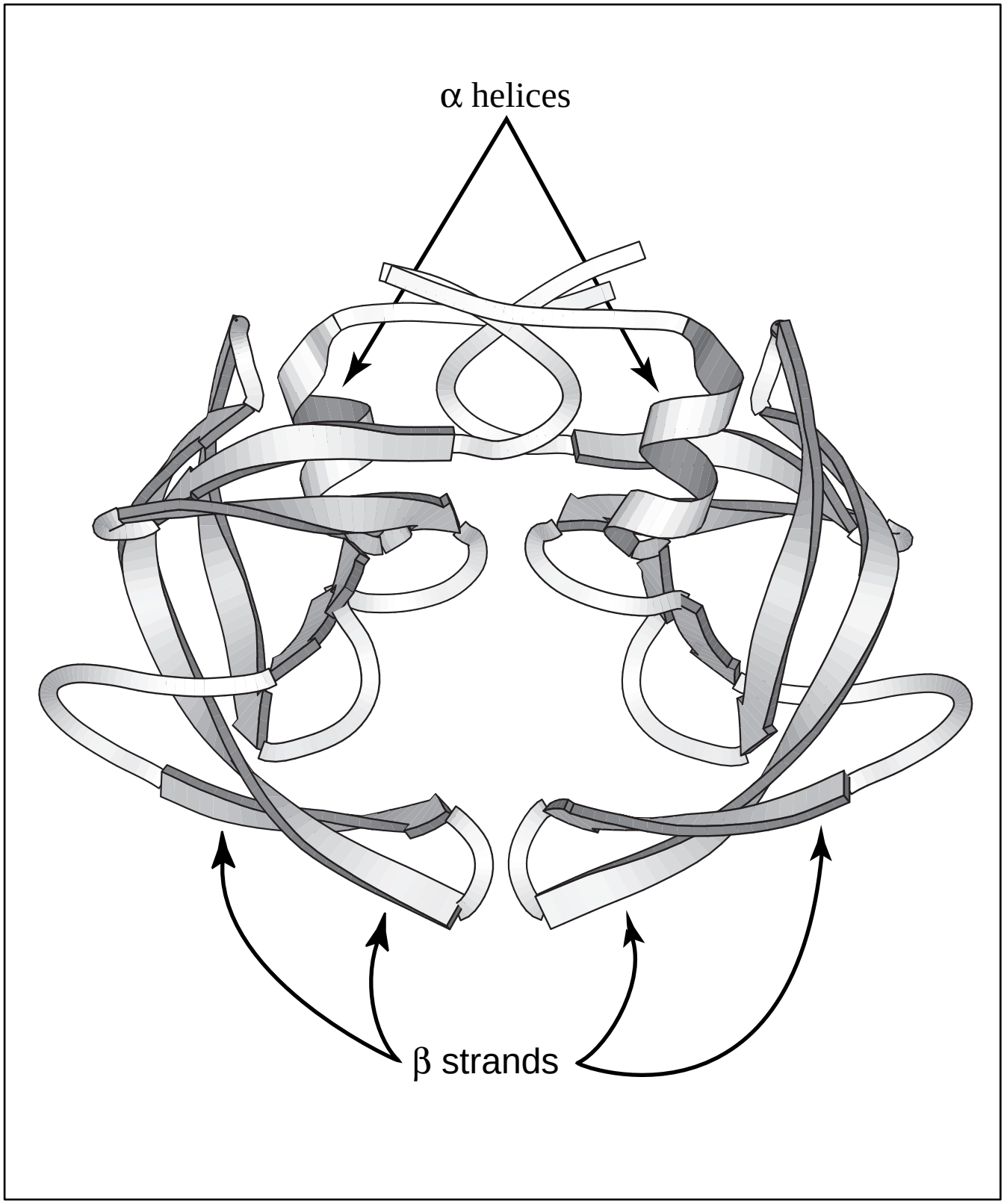

Figure 5. HIV-1 protease dimer.

Figure 6. HIV-1 protease dimer around pre-protein.

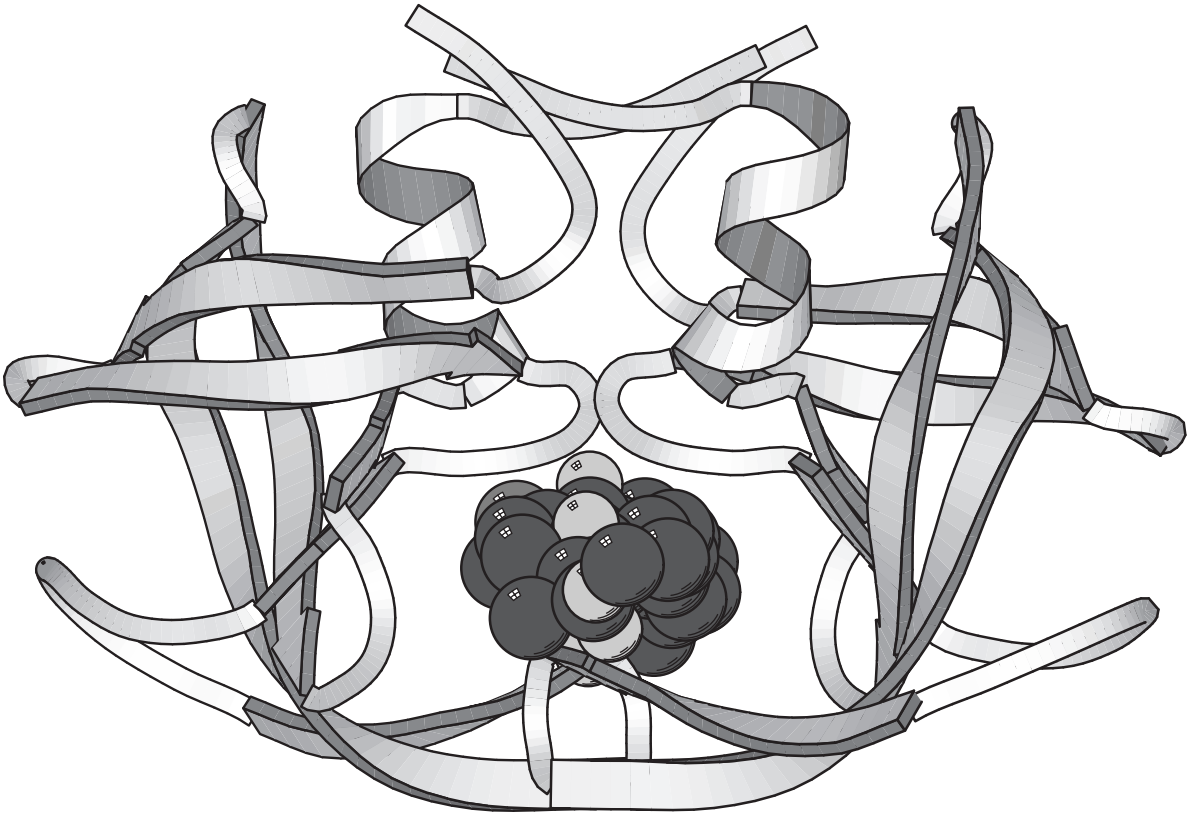

Figure 7. HIV-1 protease with inhibitor in the active site.