

BL/CH401 Lecture 11 -- Introduction to Enzymes

Part I. Background: What do enzymes really look like?

Floating in space in front of the screen at eye level was a computer-generated visual model of the receptor site within the human protein (enzyme) dihydrofolate reductase. The protein looked like a lumpy, airy, sculpture made of clouds of blue and red points aggregated into the shape of beach balls and tennis balls melded together...The colored sphere-clouds were clumped, folded and twisted into a geometrically complex pocket -- the docking site (the active site of the enzyme). Think of it as similar to the kind of 3-D puzzle found inside a lock: a proper key opens a door by solving the lock.

Quotation from **Virtual Reality** by Howard Reinhold, 1991, p. 26-27 (copyright ©1991 Howard Reinhold).

How are computers changing the way we see enzymes? Clearly, this is illustrated by the quote above from Reinhold's *Virtual Reality*. Reinhold is describing what he saw when shown the structure of an enzyme in a virtual reality machine at the Univ. of North Carolina. His description of the active site of the enzyme is much more dynamic and colorful than anything I have been able to show you even with the computer programs showing the 3-D shape of proteins. The future prospect is that virtual reality machines will bring a better picture of what proteins really look and how they interact with the small molecules they bind like their substrates.

To give another perspective on enzyme structure, here are some 3-D drawings of several proteins presented at the same scale. These drawings show the proteins at 5 million times their real size. The drawings emphasize the irregular surface of proteins. This may help you to understand what enzymes really look like.

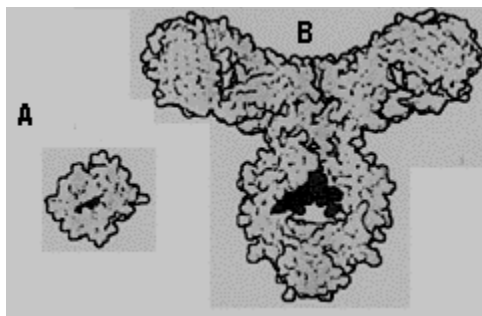


Figure 1. Cytochrome c (A) compared to an antibody (B).

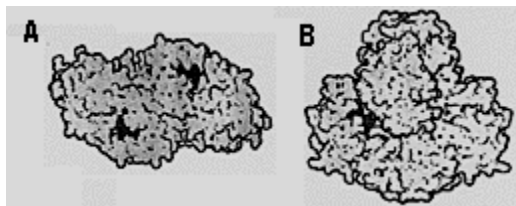


Figure 2. Two dehydrogenases: A) alcohol dehydrogenase; and B) GAP dehydrogenase. These

figures are from American Scientist 80: 460-1, 1992.

Part II. Enzyme Types

Enzymes are biological catalysts. Like all catalysts, enzymes lower the energy needed to get a reaction started. Enzymes are much generally better at accelerating the rates of reactions than non-biological catalysts.

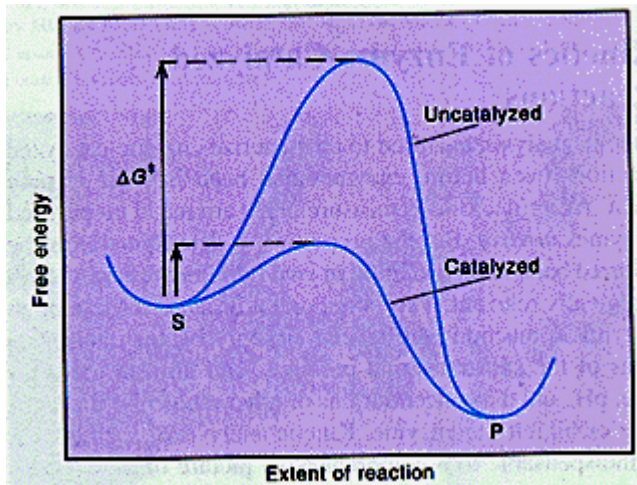


Figure 3. Diagram showing that less energy is required to get an enzyme catalyzed reaction started as compared to a non-catalyzed reaction. Figure from Zubay et al., Principles of Biochemistry copyright 1995 Brown Comm.

Enzymes have been divided into 6 classes by the International Commission on Enzyme Nomenclature. All enzymes are assigned a number (called an EC number) which defines exactly the reaction catalyzed by the enzyme. For example, trypsin is EC 3.4.21.4 since it is in class 3 (hydrolases) which work on peptide bonds (3.4) in the middle of proteins (3.4.21 are serine endopeptidases) - trypsin is the 4th entry in this subclass. Enzyme EC numbers can be looked up on the Web at <http://expasy.hcuge.ch/sprot/enzyme.ht>

These six classes are:

1. Oxidoreductases - enzymes catalyzing oxidation reduction reactions.
2. Transferases - enzymes catalyzing transfer of functional groups.
3. Hydrolases - enzymes catalyzing hydrolysis reactions.
4. Lyases - enzymes catalyzing group elimination reactions to form double bonds.
5. Isomerases - enzymes catalyzing isomerizations (bond rearrangements).
6. Ligases - enzymes catalyzing bond formation reactions couples with ATP hydrolysis.

These 6 enzyme classes can also be illustrated by the general reactions catalyzed:

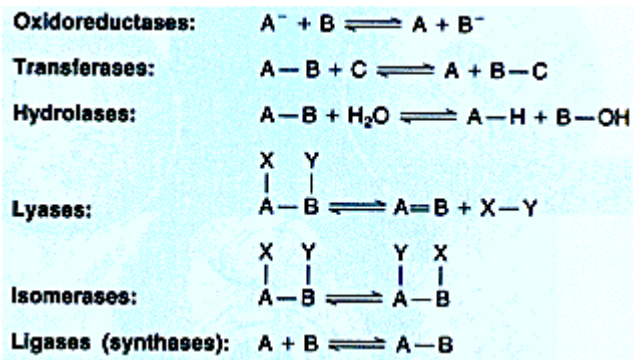


Figure 4. Model reactions of the 6 classes of enzymes. Figure from Zubay et al., Principles of Biochemistry copyright ©1995 Brown Comm.

Examples of enzymes in each class:

1. Alcohol dehydrogenase (EC 1.1.1.1)
2. Hexokinase (EC 2.7.1.1)
3. Trypsin (EC 3.4.21.4)
4. Ribulose-bisphosphate carboxylase (EC 4.1.1.39)
5. Triose phosphate isomerase (EC 5.3.1.1)
6. Tyrosine tRNA ligase (6.1.1.1)

Part III. Enzyme Additives (Cofactors) Assisting in Catalysis

Enzymes are often composed of only protein. In this case only AA side chains are used for catalysis. Some enzymes require additives for assisting with catalysis. Additives like vitamins often provide functional groups not available to the enzyme among the side chains of the amino acids.

In these cases the protein of the enzyme binds:

- Organic cofactors (Vitamins = organic cofactors)
- Metal ions (e.g. Mg²⁺)
- Nucleotides (even RNA)

The Common Cofactors (Enzyme Additives):

- Biotin aids in carboxylation reactions (carbon dioxide fixation).
- Cobaltamine (vitamin B-12) aids in alkylation reactions (methylation for instance).
- Coenzyme A aids in acyl transfers like in the tricarboxylic acid cycle.
- Flavin (vitamin B-2) aids in oxidation-reduction reactions (e.g. nitrate reductase).

- Lipoic acid aids in acyl transfers via oxidation-reduction processes.
- Nicotinamide coenzymes like NAD^+ act as independent co-substrates.
- Pyridoxal (vitamin B-6) aids in amino group transfers (provides aldehyde functional group).
- Tetrahydrofolate aids in one-carbon transfers.
- Thiamin (vitamin B-1) aids in aldehyde transfers and alpha-keto-acids decarboxylations

The complex of protein and additive is called Holo-Enzyme. When the additive is removed from the enzyme, the remaining protein part of the enzyme is called the Apo-Enzyme.

Apo-Enzyme (inactive) + Additive = Holo-Enzyme (active)

Part IV. The Active Site of the Enzyme.

Each enzyme has a unique active site.

Active site = catalytic site.

The enzyme binds its substrate(s) at the active site and the enzyme catalyzes chemical changes in the substrate(s). The types of chemical reactions catalyzed were illustrated above in Fig. 4. The binding of a substrate (NAD^+) to the active site of an enzyme is illustrated below:

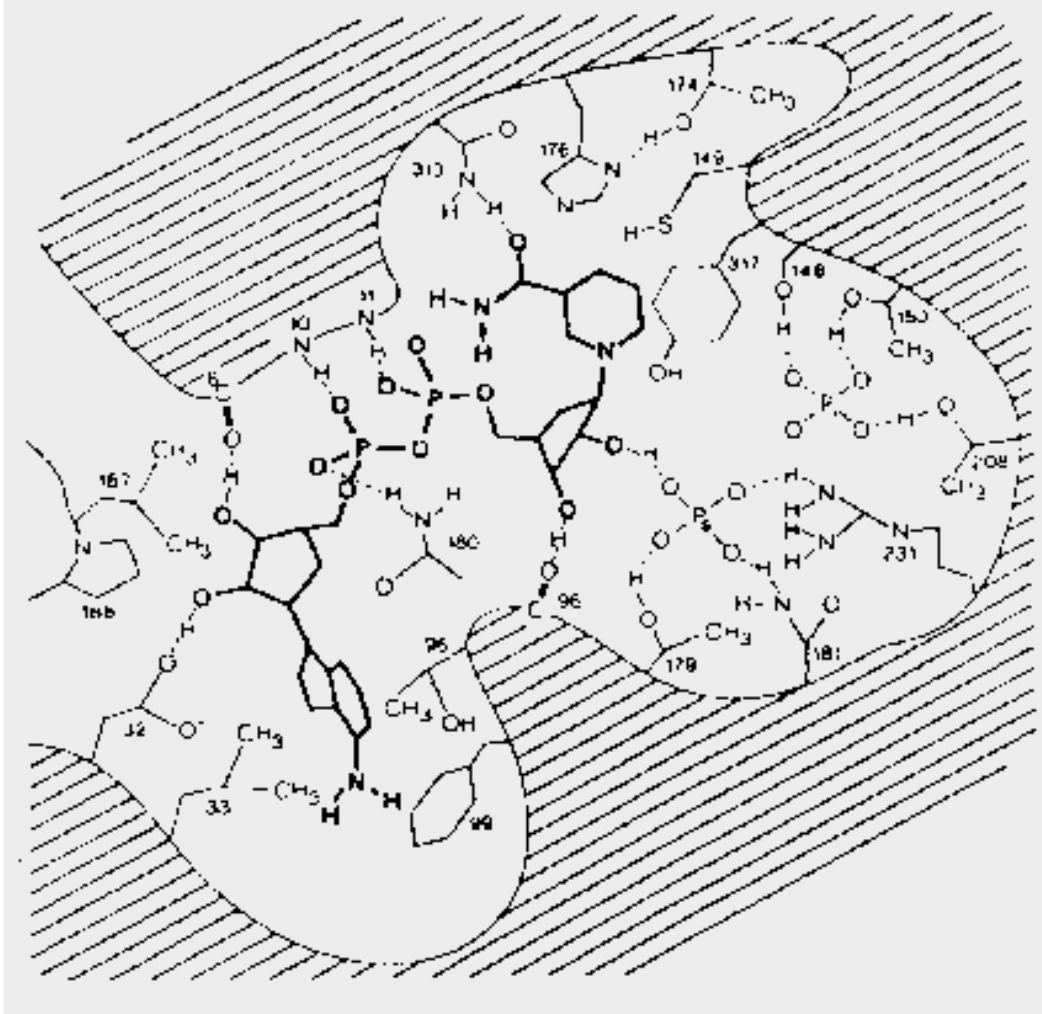


Figure 5. NAD^+ bound in the active site of GAP dehydrogenase. The NAD^+ molecule is shown in bold and the side chains of the amino acids binding it are shown projecting from the surface of the enzyme (shown as the filled in area surrounding the active site). Figure from Nature 266: 332 (1977).

Enzymes contain a large number of amino acids, but most AA side chains are used for forming the enzyme's shape. Only a few AA side chains are at the active site. These special AA side chains:

1. Bind the substrate(s) and
2. Catalyze the reaction

This concept is illustrated in the following figures by 3 different drawings of the enzyme ribonuclease which catalyzes the hydrolysis of RNA. The first view is of the 3-D shape of the enzyme with the 3 key amino acids at the active site highlighted (His12, Lys41 and His119 - numbers indicating the position of these residues in the amino acid sequence of ribonuclease). Next is a ribbon model with the 3 key amino acids shown in relation to the various secondary structure elements of ribonuclease. Last is a ball-and-stick model of ribonuclease with the same 3

amino acid side chains of the active site emphasized. A feature to try to see in these models is the groove of the enzyme which forms the active site and how the enzyme folds to bring these 3 key amino acid side chains together to form the active site.

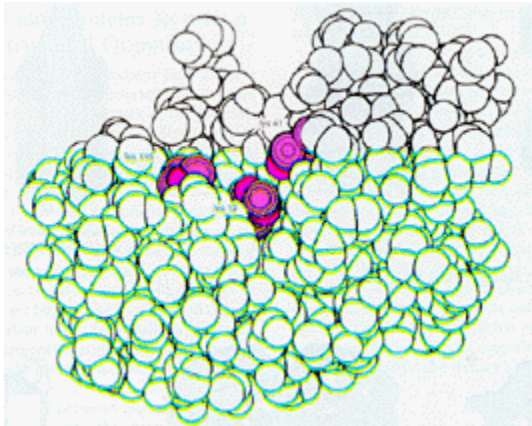


Figure 6. 3-D model of the enzyme ribonuclease with the key amino acid side chains at the active site shown in red. The active site is a deep groove at the center of this structure.

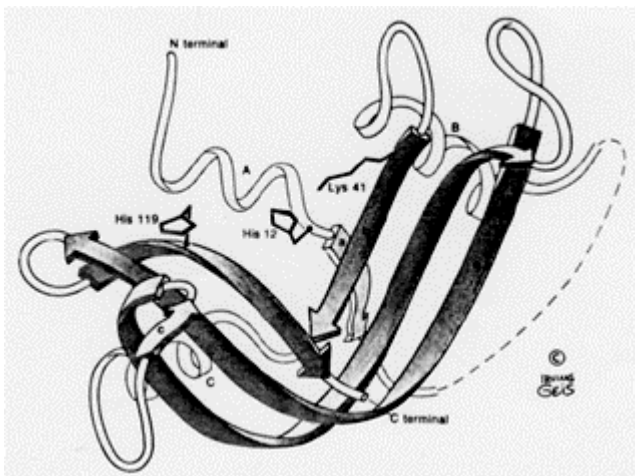


Figure 7. Ribbon model of ribonuclease which emphasizes the secondary structure of the enzyme. The key amino acid side chains are shown projecting into the active site groove. The dashed line indicates a section of the polypeptide backbone removed by proteolysis during processing of the enzyme in the intestine where it functions.

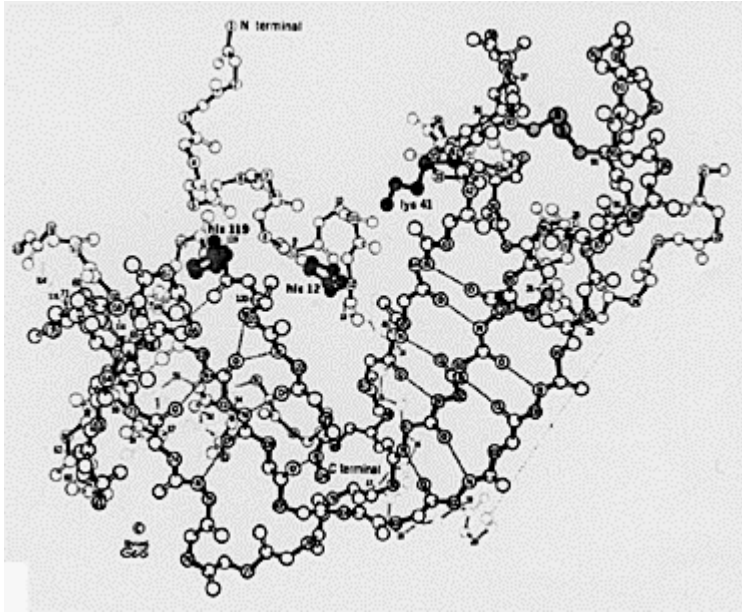


Figure 8. Ball-and-stick model of ribonuclease with the side chains of the 3 key amino acids in the active site groove shown in bold. Compare this model to the ribbon model above. These figures are from Zubay et al., Principles of Biochemistry copyright ©1995 Brown Comm.

Summary of the Active Site of Enzymes:

- Enzyme has large structure with hundreds of AA side chains but only a few are involved in catalysis.
- Each enzyme has a unique active site.
- Key AA side chains are involved in binding and catalysis in the active site.

Part V. Enzyme Framework - Why are Enzymes so Large?

We have discussed the formation of a protein's 3-D shape recall- the 4 levels of protein structure: Primary, Secondary, Tertiary, Quaternary. They make up a "Framework" to bring the AA side chains of the active site together. By bringing the AA side chains of the active site together they can act synergistically or in concert which is part of what makes enzymes very effective catalysts.

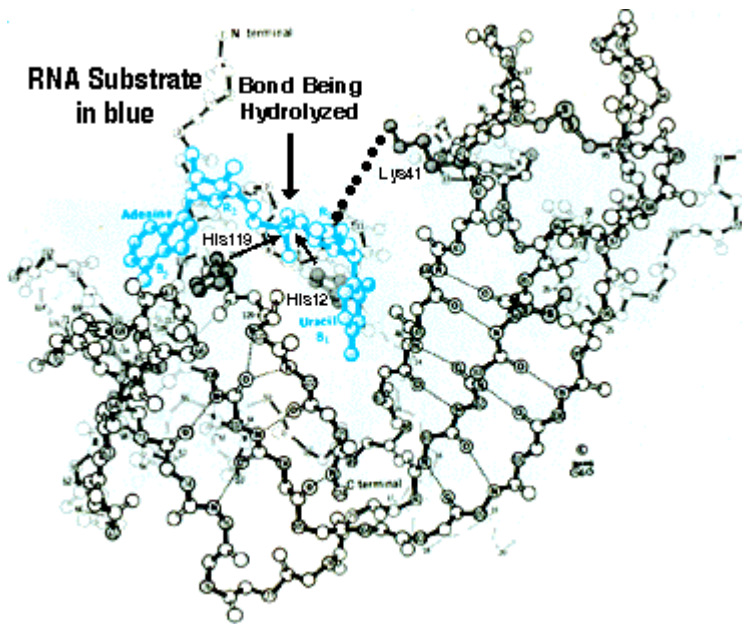


Figure 9. Ribonuclease with substrate RNA model bound in active site. His12 and His119 are involved in catalysis of the phosphodiester bond in the backbone of the RNA. Lys41 assists with binding the RNA molecule.

The active AA side chains also provide the enzyme with a high degree of specificity so that only certain substrates are bound to the enzyme's active site.

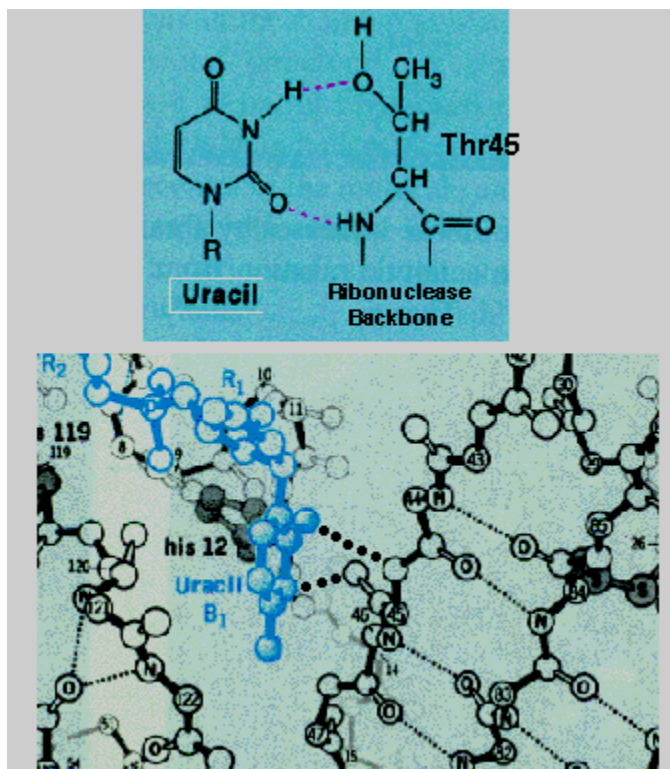


Figure 10. Model of RNA substrate bound to ribonuclease. On the top is shown the hydrogen

bonding between Thr45 and the uracil group of the model RNA substrate. On the bottom is shown a close up of the model RNA substrate bound to the ball-and-stick model of ribonuclease with the H-bonds between the substrate uracil and the key Thr45 hydroxyl group and its alpha-amino group. Compare this figure to Fig. 9 where whole enzyme is shown. Figure 9 & 10 from Zubay et al., Principles of Biochemistry copyright ©1995 Brown Comm.

KEY POINT: The concerted action of the AA side chains during catalysis also controls the formation of products so that only specific products are made and side reactions do not occur.

Part VI. How do enzymes catalyze a reaction???

One answer is: Like all catalysts, enzymes decrease the energy required to get a reaction started. This was illustrated in the first part of this lecture with an energy diagram. Below is shown a similar diagram with more detail for the energy pattern for the enzyme catalyzed reaction. First, energy is required to form the complex between the enzyme and substrate (E-S complex) which is a higher energy state than the free enzyme and substrate/product.

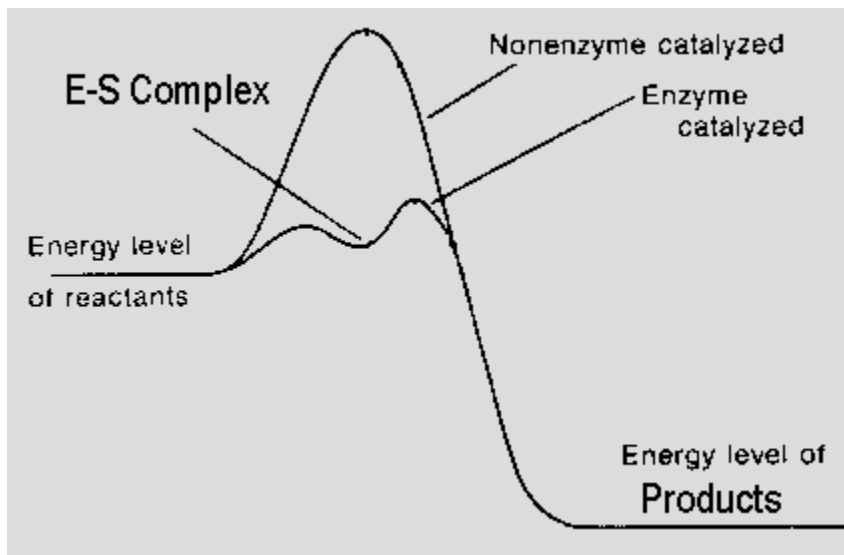
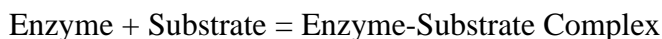


Figure 11. Diagram of energetics of enzyme catalyzed reaction versus non-catalyzed reaction.

Another answer is more difficult to describe: Enzymes use binding energy from the binding of substrates to assist in catalysis. Because the enzyme is flexible, the framework can "absorb" energy in a very efficient way and put this energy to use for assisting with catalysis.



This binding releases energy (See Figure 11 for illustration of the energy released on binding of substrate to the enzyme). However, calculations of the energy released show it is smaller than expected. The conclusion is that enzyme took up some of the binding energy. This absorbed

energy taken up by the framework of the enzyme is used by it to assist in catalysis. Thus, this helps explain why enzymes are so large - that is the enzyme has a large framework in order to use it as a resource to store binding energy made available when the substrate binds to the enzyme which it can later use to drive the formation of products. For all this energy exchange to be efficient, the enzyme must be very flexible.

Summary of Enzyme Catalysis:

- Enzymes bind substrate with great specificity
- Enzyme catalyzed reactions usually have no side products
- Enzymes use energy released when substrates bind to make their catalysis more effective.

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