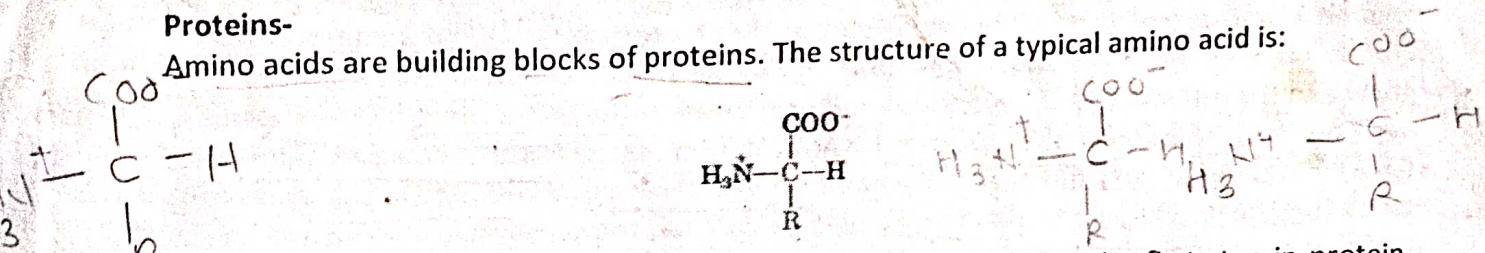
30.8.2018 – 4.8.2018

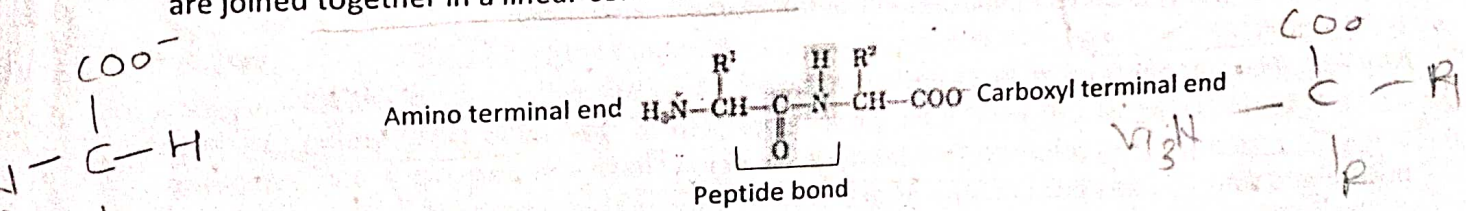
All notes have been taken &modified from:
Nelson and Cox, Lehninger Principles of Biochemistry (2004).
Stryer (7th Edition)

Proteins-

Amino acids are building blocks of proteins. The structure of a typical amino acid is:



All proteins are synthesized from a repertoire of 20 amino acids. The first step in protein synthesis is the formation of a polypeptide chain (**primary structure**), in which amino acids are joined together in a linear conformation.



The **secondary and tertiary structures, comprising the α-helix and the β-strands,** are formed due to changes in spatial conformation of the polypeptide chain or chains in a particular protein. Thisensures spatial proximity ofparticular amino acid residues essential for the protein to function effectively.

What are enzymes?

Enzymes arehighly specialized proteins that work as biological catalysts. They have extraordinary catalytic power, often far greater than that of synthetic or inorganic catalysts. Imagine a jar of sucrose/sugar left unattended on the kitchen shelf: it will not break down into CO_2 and water, but the same sucrose when consumed by an organism can be broken down effectively in the body with the help of enzymes. Thus, enzymes form the very crux of all biochemical processes occurring in any living organism. Acting in organized sequences, they catalyze the hundreds of stepwise reactions that degrade nutrient molecules, conserve and transform chemical energy, and also have the ability to build biological macromolecules from simple precursors.

History of enzymes

Much of the history of biochemistry is the history of enzyme research. Biological catalysis was first recognized and described in the late 1700s, in studies on the digestion of meat by secretions of the stomach, and research continued in the 1800s with examinations of the conversion of starch to sugar by saliva and various plant extracts. In the 1850s, Louis Pasteur concluded that fermentation of sugar into alcohol by yeast is catalyzed by "ferments." He postulated that these ferments were inseparable from the structure of living yeast cells: this view, called vitalism, prevailed for decades. Then in 1897 Eduard Buchner discovered that yeast extracts could ferment sugar to alcohol, proving that fermentation was promoted by

these molecules enzymes.

How does an enzymatic reaction work?

In order to explain the stereochemical specificity of enzymes, Alexander Ogston (1948) pointed out that there must be at least three different points of interaction between enzyme and its **substrate**, all interactions occurring predominantly in the confines of a pocket on the enzyme called the **active site.** The surface of the active site is lined with amino acid residues that bind the substrate and catalyze its chemical transformation. The conformation of an active site and placement of the amino acid residues in it is extremely crucial for the enzymatic reaction to take place. However, the active site often includes both polar and non-polar amino acid residues, creating an arrangement of hydrophilic and hydrophobic microenvironments not found elsewhere on the enzyme molecule. Hence the function of an enzyme may depend not only on the spatial arrangement of binding and catalytic sites but also on the environment in which these sites occur.

Additional components of an enzymatic reaction

Some enzymes require additional components apart from the amino acid residues in their active site, in order to complete the catalytic process. These components are of a varying nature and may be used either alone or in a combination with each other:

1. **Cofactor**- Inorganic ions (Fe^2, Mg^2, Mn^2, or Zn^2),
2. **Coenzyme**- Complex organic or metalloorganic molecule (biocytin, coenzyme A, FAD, NAD). Coenzymes act as transient carriers of specific functional group (CO_2, acyl or alkyl groups or, in case of oxidoreductase enzymes, carrier of electrons).

When a coenzyme or metal ion is tightly or even covalently bound to the enzyme, it may also be called as a **prosthetic group.**

A complete, **catalytically active enzyme** together with its **bound coenzyme and/or metal ions** is called a **holoenzyme.** In this state, the protein part of the holoenzyme (enzyme complex) is called the **apoenzyme** or apoprotein.

To understand enzyme catalysis, we must first appreciate the important distinction between **reaction equilibria and reaction rates**. The function of a catalyst is to **acceleratethe rate** of a reaction; however, catalysts do not affect the reaction equilibria (that is: the total concentration of substrate or product in any enzymatic reaction is not affected by the enzyme itself).

$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$

E= Enzyme; S = Substrate; P = Product

In the illustrative shown above, ES and EP are transient state complexes of the enzyme with the substrate and with the product respectively, that occur before the complete conversion of the substrate into a product. Virtually any biochemical reaction can be shown by the above illustrative. The bidirectional arrows indicate that the substrate and the product are interconvertible and that the reaction is reversible.

Depending upon the interactions between the substrate and the active site of an enzyme, different models of enzyme catalysis have been described over the years.

The Fischer "Lock-and-Key" Hypothesis

As early as 1890, Emil Fischer suggested that an enzyme specificity implied that the substrate fits into an enzyme askey fits into a lock, thus giving rise to the "lock-and-key" hypothesis. According to this hypothesis, all structures (substrate as well as enzyme active site) remain fixed and rigid throughout the entire binding process.Thus, in case of a lock-and-key hypothesis, the active site is rigid and structurally intact, with catalytic sites aligned and freely accessible. Therefore, a suitable reactive group whether or not a part of an intact substrate can interact with these sites causing some degree of reaction to take place.

The Koschland "Induced Fit" hypothesis

The lock and key hypothesis explains many features of enzyme-substrate complementarity and enzyme specificity; however, it does not take into account the known flexibility of proteins. Thus, Daniel Koschland in 1958, suggested that the structure of a substrate may be complementary to the active site of an enzyme only in an enzyme-substrate complex and not in a free enzyme. This hypothesis assumes that the binding of a substrate to an enzyme can bring about a conformational change in the tertiary structure of the enzyme without affecting its primary structure. Thus, the bonds formed between an enzyme and substrate may replace previously existing bonds between the binding and catalytic sites in the active site of an enzyme. Also, the presence of a substrate at an active site may result in exclusion of water molecules, making the region more hydrophobic. Since the required matching of the substrate and the active site of an enzyme occurs only after a conformational change in the enzyme, this hypothesis assumes that the nature of the active site be floppy. Thus, the enzyme wraps itself around the rigid substrate, bringing together the amino acid residues required for binding as well as catalysis. As opposed to the lock-and-key hypothesis, in case of an induced fit hypothesis the catalytic sites are not always accessible or aligned in a free (non-substrate bound) enzyme, so the risk of chance collision and reaction is minimal. This ensures a high degree of specificity. In some cases, a similar binding group may trigger off a conformational change and binding of the substrate to the enzyme, but this may not result in the catalytic groups being brought together. Thus, a non-productive binding would take place, without any successful enzymatic reaction.

An example of biochemical reaction that might take place via induced-fit mechanism is the one catalyzed by **hexokinase:**

D-Hexose + ATP $\rightleftharpoons$ D-Hexose-6-P + ADP

In this case, the cofactor ATP is bound to the enzyme hexokinase, and this bound ATP may already be hydrolyzed extremely slowly. However, a conformational change occurs only when the hexose binds to the hexokinase, thus bringing about the conversion to the product: Hexose-6-P.

Hypotheses involving strain or Transition-state stabilization

Although the lock-and-key and induced fit models can explain enzyme specificity neither suggests any direct mechanism by which the reaction can be driven forward. Substrate binding itself requires expenditure of a considerable amount of energy and although it serves a useful purpose in bringing the binding and catalytic groups together, further energy

must be supplied for the reaction to proceed. In 1930, John (J.B.S.) Haldane pointed out that if the binding energy of the substrate and the enzyme was used to distort the substrate in such a way as to facilitate the subsequent reaction, then less energy would be required for the reaction to take place. This concept was developed further by Linus Pauling in 1948.

Under the assumption that the substrate is not perfectly complementary to the active site, the substrate must be distorted in order for binding to occur. This distortion and stretching may result in weakening of a bond that is to be subsequently modified/cleaved in the substrate, thus assisting the reaction forward. This hypothesis is known as the **strain hypothesis**. Another alternative which has been proposed is the **transition state stabilization** hypothesis. This hypothesis assumes that the substrate is bound in an undistorted form, but the enzyme substrate complex possesses various unfavorable interactions. This tends to distort the substrate in a way that the enzyme-substrate complex is stabilized transiently, leading to the transition states: ES, EP (refer to illustrative on page 2) until the complete reaction occurs and the products are formed.

Enzyme specificity

Specificity of an enzyme action is determined by two independent factors: (i). the relative ability of a potential substrate to bind to the enzyme, and (ii). once bound, the relative ability to undergo a particular reaction. Only the overall rate of product formation determines the ability of an enzyme to utilize a particular substrate. In many cases of enzymatic reactions, a combination of the above-mentioned hypotheses occurs: for example, the induced-fit and transition-state stabilization mechanisms are not entirely mutually exclusive. If a precise conformational change upon formation of an enzyme-substrate complex occurs (induced-fit), the binding of the substrate could lead to the presence of a strain or stabilization of the transition state products. This will then drive the reaction forward.

Enzyme specificities are of a varying nature. Some enzymes show specificity only for one type of substrate, thus displaying **absolute specificity**; while other enzymes show specificity for a group of closely related substrates having a common chemical group, thus displaying **group specificity**. For example, **hexokinase**catalyses enzymatic reactions for all hexose sugars, while **glucokinase** catalyses enzymatic reactions specifically for glucose.In addition to chemical specificity, many enzymes may also display **stereochemical specificity**: that is, catalyses reactions only for a particular stereochemical form of the substrate (L-form or D-form).

International onion of Biochem Stry

Enzyme classification

The International Union of Biochemistry (IUB) has classified enzymes into 6 major classes depending upon the type of reaction they catalyze:

oxidoreductase

Class I- Oxidoreductases

Oxidoreductases are class of enzymes that carry out oxidation-reduction reactions.

Some examples: Catalases (Peroxidases), Oxidases, Dehydrogenases

oxygenases

Class II- Transferases

Transferases are class of enzymes that carry out the transfer of a functional group

Some examples: kinases, methyltransferases, aminotransferases

Class III- Hydrolases

Hydrolases are class of enzymes that perform hydrolysis

Some examples: amylases, proteases, lipases, nucleases

Hydrolases phosphotases

Class IV- Lyases

Lyases are class of enzymes that perform lysis

Some examples:aldolases, decarboxylases synthases

Class V- Isomerases

Isomerases are class of enzymes that result in spatial rearrangement and interconversion from one isomer to another

Some examples: enolases, racemases, mutases

enolases recemases mutases

Class VI- Ligases

Ligases are class of enzymes that bring about the ligation or joining of two molecules to form a bigger molecule.

Some examples: synthases, synthetases carboxylases

Identification of enzymes cased upon their unique identification number: Enzyme Commission (E.C.) number

The first Enzyme Commission devised a system for classification of enzymes that also serves as a unique identification number for these enzymes. These code numbers, prefixed by EC, which are now widely in use, contain four elements separated by points:

(i) the first number denotes the main class of that enzyme (Class I to class VI)

(ii) the second figure indicates the subclass

(iii) the third figure gives the sub-subclass

synthases synthetases

(iv) the fourth figure is the serial number of the enzyme in its sub-subclass

Example: Class I Oxidoreductases

The second figure (subclass) indicates the electron donor, except for subclasses 11, 13, 14 and 15.

Second figure for oxidoreductases:

1.1 denotes CH-OH as the electron donor

1.2 denotes CHO as the electron donor

1.3 denotes COOH as the electron donor

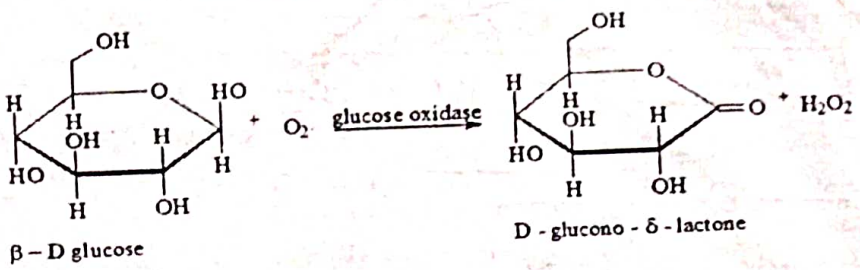
1.11 denotes H_2O_2 as the electron acceptor

The third figure (sub-subclass) indicates the electron acceptor, except for subclasses 11, 13, 14 and 15. The third figure for oxidoreductases with subclass 1:

1.1.1 denotes NAD or NADP as the electron acceptor

1.1.2 denotes cytochrome as the electron acceptor

1.1.3 denotes oxygen as the electron acceptor
Example: EC number for **glucose oxidase**which catalyzes the following reaction:



β – D glucose

D - glucono - δ - lactone

1.1.3.4, where the first digit denotes that it belongs to the class oxidoreductase, the second digit denotes that the electron donor is a CH-OH bond, the third digit denotes that the electron acceptor is oxygen, and the fourth digit is the serial number of oxidase in the sub-sub class 1.1.3.

Metabolism – A set of chemical process that occur within an organism to maintain life. These processes are described as 'metabolic pathways'.
Catabolism – Also known as **destructive** metabolism, as the name suggests: the breakdown of complex molecules into simpler molecules. In a catabolic pathway, energy is released.
Anabolism – Also known as **constructive** metabolism,as the name suggests: the assimilation of simpler molecules into complex molecules. In an anabolic pathway, energy is consumed.
Amphibolism – Comprises **both: destructive as well as constructive** metabolism. Hence, an amphibolic pathway consists of both catabolic as well as anabolic processes. In a classic amphibolic pathway: complex molecules are broken down into simpler molecules and the resultant energy is further used for construction of other complex molecules.

Some common functional groups to be encountered in metabolic pathways