“DEVELOPMENTS IN THE FIELD OF ENZYME INHIBITION: AN OVERVIEW”
Ravi Tripathi, Anchala Guglani
Assistant Professor, Department of Pharmacy, Teerthanker Mahaveer University, Moradabad, U.P.- 244001.

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Enzymes, Enzyme inhibition, disease.

For Correspondence:
Ravi Tripathi *
Address:
Assistant Professor,
Department of Pharmacy,
Teerthanker Mahaveer
University, Moradabad,
U.P.- 244001.

ABSTRACT
Enzymes are biological catalysts-they catalyze the chemical reactions that happen inside living things. Biochemical reaction occurs only when the right enzyme binds with its right substrate. Enzyme inhibition can happen when the inhibitors (structural analogs of substrate) binds to the active site of the enzyme and prevents the catalysis. Enzyme inhibitors are the most commonly therapeutic used in traditional health care system. Enzyme inhibition has recently been identified as an alternative and significant target. These are exploited in the treatment of assorted maladies such as diabetes, cancer, digestive disorders and hepatic disease. The present work has been sincerely focused on to summarize enzymes and their inhibitors recently being intensively investigated across world. At present, examples of the enzymes being intensively investigated include Histone deacetylases, N-acetylneuraminate lyase and Dipeptidyl Peptidase-4 (DPP-4).
INTRODUCTION:

1. ENZYME

Proteins can be divided into two major categories: first, structural proteins, and second, biologically-active proteins. While structural proteins mainly constitute parts of our body in form of collagen, hair, muscle, bones, tendons etc. However, biologically-active proteins, called as enzyme are responsible for the catalysis of biochemical reactions within the cells.

Enzymes are life’s great facilitators. They are responsible for the conditions required for biochemical reactions to happen fast. The general name that chemists use for a chemical entity that increases the speed of a reaction is a “catalyst.” Enzymes are biological catalysts—they catalyze the chemical reactions that happen inside living things.

Enzymes encompass only one function, and works resembling a key that fits into a lock. Biochemical reaction occurs only when the right enzyme binds with its right substrate. Usually, enzymes function at lower temperature and optimum pH and are considered to be one of the most stable catalysts in comparison to other chemicals or biological molecules. Enzymes are biodegradable, work until completely dissolved these properties make them the most eco-friendly option for industrial manufacturing.

Since enzymes never structurally constitute final product of the biochemical, hence it is quite convenient to utilize them as catalyst for the same reaction repetitively, given the right environment for the reaction.

2. Structure Of Enzyme

As enzymes are nothing but proteins, therefore the structural units of them are also amino acids. Amino acids are present in a number hundred to millions forming the shape like beads on a string. Various chemical bonds are responsible for binding of each amino acid to the next. Every enzyme persist its unique sequence of amino acids, which is determined by the genes in the cells. The vast majority of enzymes are made of only 20 different kinds of amino acid. The structure and function of any enzyme depends on the order of the amino acids, in which they are sequenced.

In most enzymes, the string of amino acids is coiled and folded thousands of times to form a highly complex three-dimensional structure, which is unique to each enzyme. The chemical interactions between the amino acids force the enzymes into three-dimensional structure, which is held collectively by various bonds among the different amino acids.

The reason behind specificity of the enzymes towards binding specific substrate is due to their unique 3-D structure. A slight change in the arrangement of the constituting amino acids results in large impact on their structural and functional properties. It has been experimentally observed that
the shuffling of just one, or perhaps a few, amino acids in the enzyme structure, results in completely different biological action of the enzymes.

Although enzymes are made up of fairly large number of amino acids but it has been found that only a specific portion of the enzyme is responsible for the entire catalysis. This specific part of the enzyme is called as active site, having very defined shape and structure. The appearance of the active site is determined by the three-dimensional structure of the enzyme. The active site selectively interacts with the substrate or part of the substrate and leads the catalysis.

3. Mechanism of Action Of Enzyme

A number of theories have been proposed for the mechanism of the action of enzymes. However, only few of them have succeeded to define possible enzymatic action, such as:

I. Lock and Key Theory

In the beginning of 19th century, Emil Fisher proposed a concept of ‘lock and key model’ to explain functioning of enzymes. In this analogy, the lock is considered as the enzyme, while the key stands for the substrate. In order to open the lock (functioning of enzyme), one need to have the exactly fitting key (substrate) for the matching lock (active site).

![Lock and Key Analogy](image)

**Fig 1: lock and key model**

Fisher explained the functioning of enzyme as, only matching key has the capability of unlocking the lock. Similarly, only substrate molecule having suitable shape will fit into the active site cavity of the enzyme, and will trigger the catalysis of the particular biochemical reaction.

II. Induced Fit Theory

Lock and key model for the enzyme action does not satisfy functioning of majority of enzyme. Further, a new modified concept was introduced by Daniel Koshland in 1958: Induced fit theory.

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According to this theory, the enzyme adapts or changes its structure according to the binding substrate. This explains why certain compounds can bind to the enzyme but do not react because the enzyme has been distorted too much. After binding of the substrate the alignment of the substrate within the active site should essentially instigate the best possible arrangement to initiate catalytic action of the enzyme. In this aspect, only rightly matching substrate is capable of inducing proper alignment in conjunction with the active site.

![Induced Fit Theory](image)

**Fig 2: Induced fit theory**

In the above depicted graphic, the substrate (magenta molecule) is shown to be submerged within structure of enzyme (green color) having placement with the active site. It can be easily implicated that the protein chains are flexible in nature and fit around the substrate.

4. **Classification of Enzyme**

Based on the type of bio-chemical reaction being catalyzed, the enzymes are classified into following classes:

I. Addition or removal of water
   a. Hydrolases
   b. Hydrases

II. Transfer of electrons
   a. Oxidases
   b. Dehydrogenases

III. Transfer of a radical
   a. Transglycosidases
b. Transphosphorylases and phosphomutases
c. Transaminases
d. Transmethylases
e. Transacetylases

IV. Splitting or forming a C-C bond
   a. Desmolases

V. Changing geometry or structure of a molecule
   a. Isomerases

VI. Joining two molecules through hydrolysis
   a. Ligases

5. Enzyme Inhibitors

After discovery of sulfonamides in mid nineteenth century enzyme inhibition has been identified as a significant target for the treatment of majority of disorders in human. Inhibition of enzyme enables control of the biochemical pathway at the critical step during the disorder condition. Besides, it also offers high degree of selectivity and efficacy of the therapeutics based on the enzyme inhibition. Therefore, the pursuit of novel enzyme inhibitors that selectively target enzymes is now the subject of intense research in the field of medicinal chemistry and chemical biology.

Enzyme inhibition can happen when the inhibitors (structural analogs of substrate) binds to the active site of the enzyme and prevents the catalysis. Compound restraint is particular and is unique in relation to the change of structure of enzyme and decrease of response rate by environmental components, for example, pH, temperature, nearness of hydrophobic compounds, detergents etc., which are non-specific. For example, consider sudden addition of an acid or base to the reaction mix which changes the pH and thereby influences the rate of the reaction. These are often confused with inhibition as they also reduce the turnover rate of enzymes.

In general, the binding of enzyme to the inhibitor is reversible but few of them bind covalently and become irreversible. The reversible and irreversible inhibitors have different kinetics. Michaelis-Menten kinetics clarifies the hindrance of enzyme in a solitary substrate complex, yet the convolution increments with the quantity of substrates. The inhibitors also depend on their homology with the substrate apart from the nature of binding site and binding affinity.

Some inhibitors even bind stronger than the natural substrate because of specific interactions and act as antagonists. Most therapeutic drug molecules act in this way. Yet there are different forms of inhibition based on the affinity of inhibitor to the enzyme and substrate.
6. **Classification Of Enzyme Inhibition**

The process of the enzyme inhibition is classified into different categories based on the competition for the binding of the substrate and inhibitor substance with enzyme. The classification follows as:

a. **Competitive inhibition**

b. **Non-competitive inhibition**

c. **Allosteric inhibition**

d. **Mixed inhibition**

a. **Competitive inhibition:**

Competitive restraint happens when the inhibitor is exceedingly homologous to the substrate particle and contends with substrate to tie to the free enzyme. So, either one of them can bind with an enzyme and not both together. In this condition, there is a need for excess substrate to overcome the competition with inhibitor. Established case for competitive inhibition is the methotrexate which represses the activity of dihydrofolate reductase to change over dihydrofolate to tetrahydrofolate.

![Fig 3: Competitive inhibition](image)

b. **Non-Competitive inhibition:**

In case of non-competitive inhibition; the inhibitor binds to the different site in the enzyme. In this way, as opposed to competitive inhibition, they can bind alongside substrate to the enzyme and here both enzyme-substrate-inhibitor complex and enzyme-inhibitor complex are dormant.

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The binding of the inhibitor with the enzyme distorts the active site cavity of the enzyme, thus making it unsuitable for the effective binding of the substrate. It is to be noted that in this case of inhibition, the inhibitor molecule does not binds with the active site or competes for the active site binding.

**E.g.**: Inhibition of CYP3C9 by Nifedipine and tranylcypromine.

**c. Allosteric or Feed Back Inhibition:**

In some very special scenarios, the product formed after the catalysis of metabolic pathways itself binds with the enzyme and inhibit enzyme activity. Such type of phenomenon is known as allosteric inhibition. This is type of self-regulatory mechanism is very common in biological systems where the formed product also acts as an inhibitor. Such an inhibitor binds to the site other than the active site, allosteric site, and inhibits the enzyme activity. This checks the binding of substrate.

**E.g.** Inhibition of hexokinase by glucose 6-phosphate
d. **Mixed inhibition:**

Mixed inhibition is the unique mode of enzyme inhibition where inhibitor binds and inactivate enzyme irrespective of its state i.e. bound with substrate or free. Usually, mixed inhibitor binds with the enzyme at their allosteric site. Such binding may result into increased or decreased affinity of the enzyme to its substrate.

**E.g.** Inhibition of the xanthine oxidase by $\text{Pd}^{2+}$.

At present, the majority of enzymes are intensively being investigated, such as:

1. **Histone Deacetylase (HDAC)**

Histone deacetylases (EC 3.5.1.98, HDAC) are a class of enzymes that evacuate acetyl gatherings ($\text{O} = \text{C} - \text{CH}_3$) from a ε-N-acetyl lysine amino acid on a histone, enabling the histone to wrap the DNA more tightly. This is important because DNA is wrapped around histone, and DNA expression is regulated by acetylation and de-acetylation. Its action is opposite to that of histone acetyltransferase. HDAC proteins are currently likewise called lysine deacetylases (LDAC), to depict their capacity as opposed to their objective, which additionally incorporates non-histone proteins.

Histone tails are normally positively charged due to amine groups present on their lysine and arginine amino acids. These positive charges facilitate the histone tails to communicate with and unite to the negatively charged phosphate groups on the DNA spine. Acetylation, which occurs normally in a cell, neutralizes the positive charges on the histone by changing amines into amides and decreases the ability of the histones to bind to DNA. This diminished binding permits chromatin development, allowing hereditary transcription to happen. Histone deacetylases remove those acetyl groups, increasing the positive charge of histone tails and encouraging high-affinity binding between the histones and DNA backbone. The increased DNA binding condenses DNA structure, preventing transcription.

Histone deacetylase is involved in a series of pathways within the living system, such as:
- Environmental information processing; signal transduction; notch signaling pathway
- Cellular processes; cell growth and death; cell cycle
- Human diseases; cancers; chronic myeloid leukemia
- Histone acetylation assumes a vital part in the control of gene expression.

**Histone deacetylase inhibitors (HDACi)**

Histone deacetylase inhibitors exert a diverse set of biochemical actions alone, or in combination, which further potentially induce anticancer activities.
HDACi works by altering transcription of proteins and induce apoptosis of the infected cells. The inhibited proteins are critical for the cancer cell metabolic pathways. In addition, HDACi are also involved in modulating of the tumor cells environment and also the immune milieu, leading to the antitumor effect. Along with some corepressors, HDACs deacetylate lysine residues of the histone terminus. On the contrary, histone acetyl transferase acetylates histone. These two enzymes regulate acetylation status of the histone.

![Mechanism of action](image)

**Fig 6: Mechanism of action**

- **Classification of HDACi:**

  Pharmacophore structure of HDACi consists of a cap, linker moieties with connecting units and chelating group that binds with Zn$^{+2}$ present in the active site of the receptor. Based on the chemical structure and ability to inhibit HDAC, histone deacetylase inhibitors have been classified:

  I. Hydroxamate
  II. Cyclic peptides
  III. Aliphatic acids and
  IV. Benzamides

  Hydroxamate class includes compound trichostatin A, which has been identified as potential HDAC inhibitor. TrichostatinA (TSA) is found to be exist naturally. Another, important chemical compound having HDAC inhibitory activity is Vorinostat, which was earlier endorsed by USFDA for the treatment of cutaneous T-cell lymphoma. Trichostatin A and vorinostat are pan-HDAC inhibitors.
Second class, the cyclic peptides are the most investigated compounds for their activity against HDAC enzyme. These compounds are structurally very complex. Depsipeptide, apicidin, and cyclic hydroxamic acid derivatives are the important examples of this category. Among the other compounds of this class, Depsipeptide is most important member, which was approved for the treatment of cutaneous T-cell lymphoma in November, 2009.

Butyrate and the phenylbutyrate are agents belonging to the class third of the classification; aliphatic acids. These compounds possess feeble HDAC inhibitory activity, and so are of least importance. SNDX-275 and MGCD0103 are the HDAC inhibitor belonging to the class benzamide.

➢ **Therapeutic Use of HDACi:**

HDAC inhibitors find their application for treatment of variety of human diseases, such as:

- Rubinstein-Taybi syndrome
- Huntington’s disease
- Rett syndrome
- Friedreich’s ataxia
- Anemia
- Renal failure etc.

2. **N-acetylneuraminic lyase (NAL)**

N-acetylneuraminic lyase catalyzes the breakdown of N-acetylneuraminic acid (sialic acid, Neu5Ac) to pyruvate and N-acetyl-D-monoamine. The enzyme assumes an essential part in the direction of sialic acid digestion in Escherichia coli. Sialic acid is toxic to E. coli either as the free sugar or in the activated cytidine 5’-monophosphorylated form. Neu5Ac lyase is induced by sialic acid and is able to regulate intracellular levels of the sugar. This control is as effective for sialic acid of biosynthetic origin as it is for exogenous sialic acid which may accumulate inside the bacterial cell. Neu5Ac lyase has been found in pathogenic as well as non-pathogenic bacteria and in mammalian tissues.

➢ **Sialic acids in human health and disease**

Sialic acid-rich glycoprotein’s (sialoglycoproteins) bind selectin in humans and other organisms. Metastatic cancer cells often express a high density of sialic acid-rich glycoproteins. This overexpression of sialic acid on surfaces creates a negative charge on cell membranes. This creates repulsion between cells (cell opposition) and helps these late-stage cancer cells enter the blood stream. Given their negative charge and hydrophilicity, sialic acids add to the biophysical components of different biological systems. For example, the negative charge on human erythrocytes and other cell types provides charge repulsion, preventing unwanted interactions of
cells in the blood circulation. The thickness of sialic acids in the glomerular basement membrane and on the foot-procedures of podocytes seems basic in keeping up the typical filtering capacity of the organ, and broadened polysialic acid chains can affect neuronal plasticity. The luminal surface of the vascular endothelium is also very heavily sialylated.

At the level of molecular physiology, sialic acids can tweak the half-life of a few proteins in the course, particularly under pathological conditions, for example, infections with sialidase-expressing bacteria (see fig. underneath).

Numerous microorganisms also utilize sialic acid in their biology, despite the fact that this is normally constrained to bacteria inhabiting in higher creatures. A large portion of these join sialic acid into cell surface components like their lipopolysaccharide and capsule, which helps them sidestep the innate immune response of the host. Other bacteria basically utilize sialic acid as a decent supplement source, as it contains both carbon and nitrogen and can be changed over to fructose-6-phosphate, which can then enter focal metabolism.

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**Fig 7: Roles of Sialic acids in various biological processes**
Table No. 1 Examples of pathogens that express sialic acid on their surfaces

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Major disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sialic acid synthesized by pathogen</strong></td>
<td></td>
</tr>
<tr>
<td>Neustria meningitides B</td>
<td>Meningitis</td>
</tr>
<tr>
<td>Escherichia coli K1</td>
<td>Neonatal meningitis</td>
</tr>
<tr>
<td>Group B Streptococcus</td>
<td>Neonate and infant infections</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>Enteritis, Guillain – Barre Syndrome</td>
</tr>
<tr>
<td><strong>Host sialic acid taken up by pathogen</strong></td>
<td></td>
</tr>
<tr>
<td>Hemophilus influenza</td>
<td>Respiratory infections</td>
</tr>
<tr>
<td>Hemophilus ducreyi Chancroid</td>
<td></td>
</tr>
<tr>
<td><strong>Host sialic acid transferred by trans-sialidase</strong></td>
<td></td>
</tr>
<tr>
<td>Trypanosoma cruzi</td>
<td>Chagas disease</td>
</tr>
<tr>
<td>Corynebacterium diphtheriae</td>
<td>Diphtheria</td>
</tr>
<tr>
<td><strong>Host CMP-sialic acid used by sialyltransferase</strong></td>
<td></td>
</tr>
<tr>
<td>Neisseria gonorrhoea</td>
<td>Gonorrhoea</td>
</tr>
<tr>
<td>Neisseria meningitidis group A</td>
<td>Meningitis</td>
</tr>
<tr>
<td><strong>Source of sialic acid unknown</strong></td>
<td></td>
</tr>
<tr>
<td>Sporothrichiumschenkii</td>
<td>Skin infection</td>
</tr>
<tr>
<td>Aspergillus fumigates</td>
<td>Opportunistic infections</td>
</tr>
</tbody>
</table>

- **N-acetyleneuraminlate lyase Inhibitor:**
  Sialic acids play pivotal role in various biological processes, such as immunological regulation, bacterial & viral infestation, cancer metastasis, etc. Taking into consideration of this fact, enzymes catalysing sialic acid, such as neuraminidases, lyases, etc., are being investigated as novel drug-targets for antimicrobial activities. 4-oxo analogues of N-acetyl-D-neuraminic acid have been reported having NAL inhibitor action. Further, Barnett et al. reported NAL enzyme can be inactivated by compounds, such as bromopyruvate and chloropyruvate.

- **Mechanism of action of NAL inhibitor:**
  N-acetyleneuraminate lyase (NAL; EC 4.1.3.3) catalyzes the breakdown of N-acetyleneuraminic acid (sialic acid) into N-acetyl-D-mannosamine and pyruvate via an intermediate Schiff base.
In bacteria, NAL is involved in the catalysis of sialic acid, which breaks sialic acid into components rich in carbon and nitrogen. Bacteria use these degraded products as a source of their nutrition. Thus, bacteria use NAL to feed themselves. This renders N-acetylneuraminate lyase a potential target for antibacterial activity against pathogenic bacteria. Inhibition of NAL can cause severe starvation of bacteria, leading up to their death.

➢ **Therapeutic Use:**
   - Antibiotic
   - Antiviral
   - Antimicrobial
   - Antifungal

3. **Dipeptidyl Peptidase-4 (DPP-4)**

Dipeptidyl peptidase-4 (DPP4) is a multifunctional protein that exerts biological activity through pleiotropic actions including: protease activity, association with adenosine deaminase (ADA), interaction with the extracellular matrix, cell surface coreceptor activity mediating viral entry, and regulation of intracellular signal transduction coupled to control of cell migration and proliferation. The complexity of DPP4 action is amplified by the panoply of bioactive DPP4 substrates, which in turn act as elegant biochemical messengers in multiple tissues, including the immune and neuroendocrine systems. Biological interest in the DPP4 enzyme has heightened after the approval of highly selective DPP4 inhibitors for the treatment of type 2 diabetes. Several excellent reviews have highlighted results of clinical trials using DPP4 inhibitors to treat type 2 diabetes; others have compared.

➢ **Dipeptidyl Peptidase-4 (DPP-4) Inhibitors**

DPP4 was initially characterized as a modulator of T-cell activation and proliferation. Observations that DPP4 levels and activity were elevated in T cells of patients with autoimmune disorders and inflammatory conditions including rheumatoid arthritis led to evaluation of DPP4 inhibitors for treatment of immune disorders involving aberrant T-cell function. However, interpretation of these
studies was complicated by observations that catalytic activity was not required for DPP4 to mediate its effects on T-cell function. Since DPP4 expression is up-directed in numerous cancers and partners with extracellular lattice proteins, the results of DPP4 hindrance were assessed in T-cell malignancies and solid tumor metastases. A nonselective inhibitor for DPP4, FAP, DPP8, and DPP9 (Val-Boro-Pro, PT-100, talabostat) was studied in clinical trials for the treatment of solid tumor malignancy and advanced stage non small cell lung cancer; the available evidence did not reveal a sufficiently robust therapeutic response to merit further clinical development. Furthermore, treatment of DPP4 null (Dpp4−/− or Cd26−/−) mice with PT-100 demonstrated stimulation of cytokine and chemokine production and a reduction in tumor incidence, suggesting that inhibition of DPP4 is not the main molecular target for these actions of PT-100. Many of the first-generation DPP4 inhibitors exhibited potent actions in the immune system and modified neoplastic cell growth, yet were subsequently found to exhibit nonselective activity independent of their actions to inhibit DPP4.

- **Mechanism of Action of DPP-4 Inhibitors**

Understanding the mechanisms through which DPP4 inhibitors exert diverse metabolic actions requires assessment of the selectivity of these agents and rigorous evaluation of evidence linking changes in levels or molecular forms of a candidate DPP4 substrate to actions emanating from administration of a DPP4 inhibitor in vivo. As outlined and discussed herein, a large number of peptides are cleaved by DPP4 in vitro, and many of these peptides are also cleaved by DPP4 in vivo. Furthermore, DPP4 inhibitors modify the relative levels of intact vs cleaved substrates, which may exhibit varying affinities for structurally related receptors further complicating assignment of mechanistic roles for key peptide substrates in transducing actions arising from DPP4 inhibition. In the following sections, we describe experiments supporting the importance of DPP4 inhibitor selectivity and critically evaluate evidence linking changes in levels of key substrates and metabolites to metabolic and glucoregulatory activities ascribed to DPP4 inhibition. Wherever possible we highlight data generated using selective antagonists or mouse genetics to link the presence or absence of peptide action to therapeutic actions ascribed to DPP4 inhibitors.

- **Example-**
  - Nesina (Alogliptin)
  - Januvia (Sitagliptin)
  - Onglyza (saxagliptin)
  - Tradjenta (Linagliptin)
These drugs are an oral diabetes medicine that helps control blood sugar levels. It works by regulating the levels of insulin in body produce after eating.

- **Therapeutic Use:** Antihyperglycemia. Treatment of diabetes mellitus type-2.

**CONCLUSION:**

Enzyme inhibitors are the most commonly therapeutic used in traditional health care system. Enzyme inhibition has recently been identified as an alternative and significant target. These are exploited in the treatment of assorted maladies such as diabetes, cancer, digestive disorders and hepatic disease. This project work has been sincerely focused on to summarize enzymes and their inhibitors recently being intensively investigated across world.

Enzyme inhibition is specific and is different from the alteration of structure of enzyme and reduction of reaction rate by non-specific environmental factors. Therefore, a vast research for the discovery and development of medicinal compounds having potent inhibitory activity on enzymes, like HDAC, DPP-4 etc., are the matter of intense research these days. In last few decades, enzyme inhibitors such a Zanamivir, Glitins, Vorinostat etc. have been successfully developed and have been launched in the market for the treatment of various diseases.

**REFERENCES:**


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