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Insights into Flavoenzymes: Structure, classification and potential biotechnological applications

Huma Aslam and Sikander Ali *

Institute of Industrial Biotechnology (IIB), GC University Lahore, Pakistan.

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Abstract

Flavoenzymes are the diverse enzymes that use the flavin cofactors for their catalysis. They are widespread from bacteria to humans and have various biological role. This review provides a comprehensive overview about the flavoenzymes, classification, catalysis mechanism and their applications. The transfer of electrons between the flavin molecule which possess basically the isoalloxazine chromophore comprises of 7,8-dimethylbenzene moiety and the hydrophilic pyrimidine ring combine to form the amphipathic molecule, and the substrate which forms the valuable products by undergone chemical transformation which also provides basis for their classification. Here mainly, dehydrogenases, flavoprotein oxidases, monooxygenases and reductases classes are explained with examples along with their many physiological roles, showcasing their involvement in essential cellular functions. However, some additional redox centers flavoproteins, metal-containg, flavocytochromes and disulphide oxidoreductases are also mentioned here. Beside that they have many other tremendous applications including in disease diagnosis, treatments, novel drugs and therapeutic approaches for various diseases, biosensors, industrial, various biotechnological applications and some recent achieved advancements are discussed in detail. This study aims to advance knowledge of these unique enzymes by providing an extensive analysis of the classification, processes, and uses of flavoenzymes. Further study of flavoenzyme catalysis is required to unveil exciting avenues for future study and invention.

Keywords: Flavoenzymes; Flavin cofactors; Isoalloxazine ring; Classification; Dehydrogenases; Flavoprotein oxidases; Applications

1 Introduction

On a broad sense, flavin is used for the group of the compounds that possess heterocyclic isoalloxazine chromophore. The first flavin molecule was identified in the biological system of yeast in early 1930s which was discovered as 1st flavoenzyme, functioning as part of Old Yellow Enzyme (OYE) in brewer's bottom yeast by Warburg and Cristian in 1932. Later on, OYE has evolved into the prototypical member of large family of flavoenzyme and hundreds of flavoenzymes were identified and characterized in various biological system sources [1]. From microorganisms to mammals, they perform the vital functions maintaining the life. They are involved in many physiological activities' and secondary and xenobiotic metabolisms, antibiotics and metabolism preparation and in signaling. For example, an enzyme, glutathione reductase (GR) found in the *Streptococcus pneumoniae* important for detoxification of O_2 and N_2 as well as for intracellular metal tolerance to ions like zinc [2]. The appropriate knowledge of the flavoenzymes is very helpful in number of applications as biocatalyst, drug discovery and synthesis and biosensing tools [3]. In each system, they perform the vital functions maintaining the life.

This flavin possess yellow color in its oxidized form. It also exhibits other colors as blue, green depending on its electronic or ionic state. Hence, they named so red flavosemiquinone, blue flavosemiquinone depending on these states.

* Corresponding author: Huma Aslam and Sikander Ali

So, when these are bound to enzymes, flavoenzymes, they can use these lights as in DNA photolyase. Many bacteria illuminate due to these flavin properties [4]. Isoalloxazine is the main part of flavin which humans can't prepare themselves derived from the riboflavin, also known as vitamin B_2 which is needed for the activity of the flavoenzyme in the respiratory chain [5]. And these flavoproteins are in the form of the flavocoenzyme, mostly FMN or FAD acting covalently linked to the apoenzyme. The reaction occurs between the flavin nucleus the isoalloxazine ring system, and substrate molecules. It has been recently investigated that it is being attached to the apoprotein dual covalent bonds. This is autocatalytic process which is very helpful in for the stability, active site saturation and flavin activation/modification and its catalytic mechanism [6].

Riboflavin-based coenzymes help in catalyzing the reaction by carrying out oxidation and reduction (electrophilic in the oxidized state and nucleophilic in the reduced state) of the substrate in number of biosynthetic pathways. Usually, they do oxidation of the amine and alcohols while olefins get desaturation. The covalent adduct is formed on C4a and N5 sites which is newly discovered which is associated with direct oxygenation of the substrate. This is also considered as the place of entry for electrons from the substrates to reduced flavin. However, these centers are so vast that they can't be foreseen from the sequence and structure analysis [7]. Indeed, when more genome of the microbe is sequenced results in ORFs in gene clusters, it would result new biosynthesis pathways for flavoenzyme catalyst. However, it is also important to be noted that flavoenzymes (utilizing flavin as a substrate) are used in secondary pathways [8].

1.1 Classification

Flavoenzymes are very selective, efficient and controllable in addition to versatility enable them to use as the biocatalyst. They are considered as the 2% of the biological catalyst [6]. And this provides the basis for classification of flavoenzymes not only on the basis of the substrates and reactions catalyzed but also on the number of electrons oxidized or reduced in redox reactions being catalyzed by flavoenzymes.

There is more than one way to classify the flavoenzymes on the basis of their catalytic mechanism and other factors as reaction catalyzed. It includes to classify them on the basis of the EC number system, and the ability to use O_2 as acceptor and redox center nature and the type of reaction is catalyzing [9]. Other urbane way is based on the addition of the folds, its sequence and function. In history, they were simply classified as the "simple" and "complex" flavoenzymes. Later are those flavoenzymes that uses other cofactor as metal ions, heme beside the flavin. However, they both share the common catalytic cycles that both oxidation and redox reaction occurs comprising of the reduction of the flavin and then its reoxidation [9]. Table 1 depicts flavoenzyme with one electron-reduced classification that can also be characterized as m/n where m and n show the number of electrons transferred in reductive and oxidative half reactions, respectively, as shown in table. But the out of the 1/1, 1/2, 2/1, and 2/2, 1/2 category is not known present [4].

Table 1 One electron-reduced-m/n classification system

Classification	Examples
Class 2/2	Transhydrogenase NADPH-thioredoxin reductase d-Lactate dehydrogenase Dehydrogenase / oxidase d-Amino acid oxidase Acyl-CoA oxidase Dehydrogenase / oxygenase Phenol hydroxylase Bacterial luciferase
Class 2/1	Dehydrogenase / electron-transferase Acyl-CoA dehydrogenase Ferredoxin-NADP reductase
Class 1/1	Pure electron-transferase Flavodoxin DNA photolyase

Differences in the protein environment surrounding the flavin's isoalloxazine ring have allowed development to produce a wide range of flavoprotein active sites and catalytic mechanisms as already mentioned. However, with the development of molecular sciences, there will be other ways to find their function. Figure 1 shows the copyright 1997 by Ludwig et al., reproduced with permission from the Protein Data Bank (PDB) with 2FOX entry.

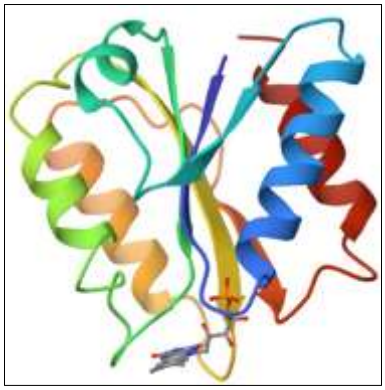

Figure 1 Structure model of flavodoxin: semiquinone from *Clostridium beijerinckii*

There are some classifications of common flavoenzymes on the basis of the versatility and specificity with its known function.

Dehydrogenases remove the hydrogen from the organic substrate and transfer them to quinones and electron transfer proteins. These type of the flavoenzymes are very common and mostly are present in the mitochondria performing their function. Any malfunctioning can result in the neurobehavioral deficits. Acyl-CoA dehydrogenase performing function by oxidation of fatty acids in mitochondria and L-galactono-1,4-lactone dehydrogenase presents in the plant performing vitamin C formation are some examples [6].

Flavoprotein reductases transfer the electron from the NAD(P)H to the substrate or other electron acceptor molecules. Flavoenzymes with this catalytic function have many important biological functions. A vital housekeeping enzyme that regulates the amount of iron in the blood is NADH: cytochrome b5 reductase (EC 1.6.2.2), important in microorganisms which helps in illumination so many examples are there [6].

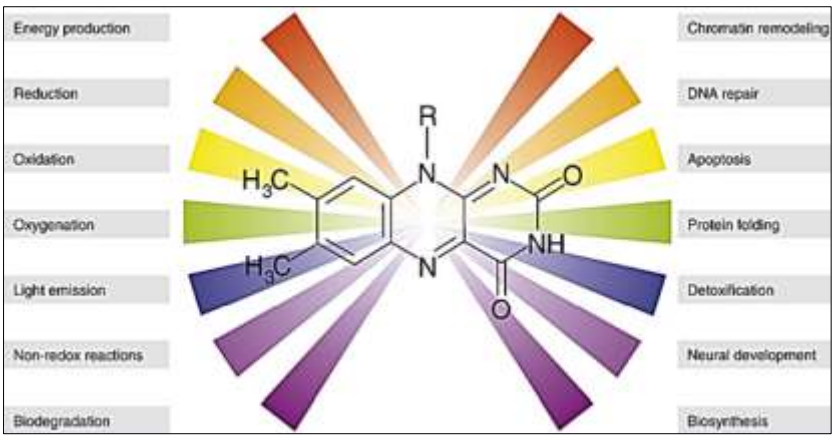

Figure 2 Biological functions of flavoenzymes

Flavoprotein oxidases regulates the changing of single bond to the double bond of the substrate. Here the reduced flavin produced during the reaction is taken by molecular oxygen to form H_2O_2. This H_2O_2 is used in food applications and diagnosis and production of improved enzyme variants in labs. This reactive oxygen species is also useful in cancer and possibly control the harmful chemical compounds production when enzymes present in low numbers are used as D-amino acid oxidase. This has many applications which is explained later on [6].

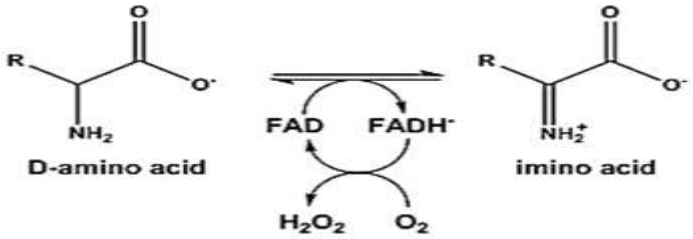

Figure 3 At first flavoenzymes bind with the substrate and FAD coenzyme. Electrons are moved from D-amino acids to FAD reducing to the FADH2. The molecular oxygen (O_2) oxidized the $FADH_2$ which accepts the electrons and become reduced to the water (H_2O). the imino acid product is released in the end and the FAD coenzyme is ready to participate in another reaction cycle

Monooxygenases which donate the electrons from NAD(P)H to the oxygen atom associated with the substrate. They are present in plants, mammals and microorganism. They perform different biological functions as breaking the lignin (aromatic hydroxylases like p-hydroxybenzoate3-hydroxylase) and formation and detoxification of plant hormones. They have significant role in the synthesis of antibiotics, antitumor drugs, and other natural substances. Flavoprotein epoxidases which is the class of the monooxygenases transmit the oxygen to C=C forming epoxide just like the heme iron monooxygenases. It is crucial in the formation of a wide variety of chemical compounds, including pharmaceuticals, agrochemicals, and industrial chemicals. Examples include styrene monooxygenase, which catalyzes the epoxidation of styrene, and p-cumate epoxidase, which catalyzes the epoxidation of p-cumate [6].

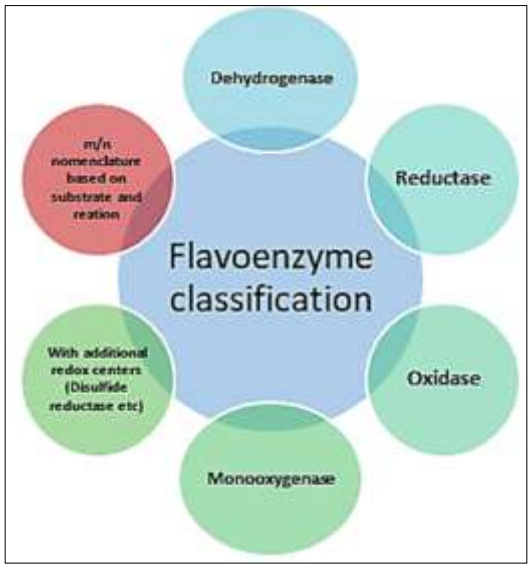

Figure 4 Classification of Flavoenzymes

1.2 Flavoproteins with additional redox centers

Flavoprotein disulfide oxidoreductases have disulfide in association with flavin the thiols active site. The flavin interacts with a pyridine nucleotide during catalysis, and then the flavin transfers electrons to the redox active disulfide/dithiol, which then interacts with the second substrate often, a disulfide or dithiol serves as the second substrate, allows reversibly. They either work in the opposite direction, producing $NAD(P)^+$and a reduced (dithiol) product, using a dithiol substrate and NAD^+. The enzymes NADH peroxidase and NADH oxidase, which are linked to disulfide oxidoreductases, each contain a single redox-active cysteine is the example of this enzyme type [10].

Heme-containing flavoproteins, also known as flavocytochromes, are a class of proteins that contain both a heme group and a flavin cofactor. The heme group acts as an electron carrier, while the flavin cofactor is involved in the transfer of electrons to or from the heme. These proteins are involved in a wide range of biological processes, including electron transfer, oxygen transport and storage, and reactive oxygen species production. Examples are cytochrome P450 enzymes, involved in the metabolism of drugs and other foreign compounds in the liver, and mitochondrial cytochrome c oxidase, involved in the final step of cellular respiration [9].

Metal-containing flavoproteins contain both a flavin cofactor and a metal ion, such as iron, copper, or zinc. The metal ion can participate in a variety of chemical reactions, including electron transfer, oxygen binding and activation, and catalysis of redox reactions. For example, includes the ferredoxin-NADP+ reductase contains a (2Fe-2S) iron-sulfur cluster and a flavin adenine dinucleotide (FAD) cofactor. They are involved in a wide range of biological processes, including energy production, oxidative stress response, and signal transduction. They are also important targets for drug development, as they play critical roles in a variety of diseases, explained later on [9].

1.3 Catalytic mechanism of flavoenzyme

Understanding the catalytic mechanism of flavoenzymes is important for developing new drugs and therapies that target these enzymes. Flavoenzymes have 7,8-dimethylbenzene moiety and the hydrophilic pyrimidine ring combine to form the amphipathic molecule, reactive isoalloxazine ring system making them extraordinary specific and versatile. It is already stated that flavocoenzyme are strongly either covalently or non-covalently bound to apoprotein. The redox potential for flavin's two-electron reduction is around 2200 mV but can vary from 2400 mV to 160 mV, with positive charges raising the potential and negative charges or hydrophobic environments lowering it [11, 12]. This is due to the fast self-oxidation of the FMN and FAD in nature. However, in 5-10% of the flavoenzyme, the isoalloxazine ring is covalently attached to His, Cys or Tyr in polypeptide chain, which also increases the redox potential. The NAD(P)H is used as the electron source by most of the enzyme [6].

Flavoenzymes catalyze the reaction in two half reactions. In first, reduction half reaction takes place in which substrate is oxidized while the bound flavin reduced. While in the second, reoxidation of the reduced flavin back to the oxidized form reducing the cosubstrate, called as reoxidative half reaction [6]. Depending on the redox state of the flavin due to ring system, it covers the large range of the reaction first as oxidized, one electron-reduced (semi quinoid, FlH, in which substrate oxidation and reductive half-reaction, followed by reoxidation of the reduced flavin and substrate reduction in the reoxidative half-reaction), and two electron-reduced, fully reduced, dihydrofavin (FlH2) states or oxygen as oxidant [13,14].

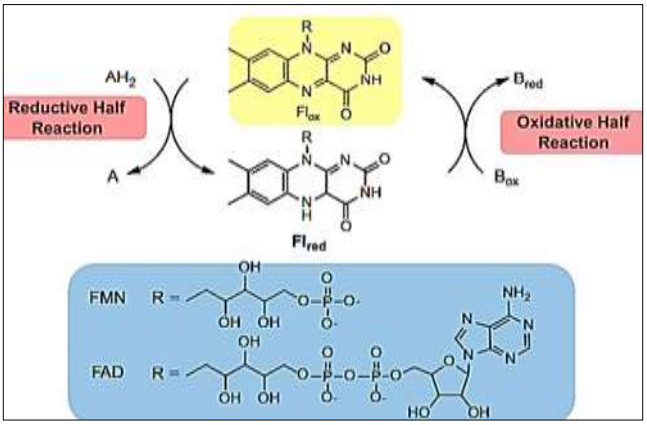

Figure 5 Two half reactions of flavoenzymes: first reductive half reaction and second oxidative half reaction

The reaction catalyzed by the flavoenzymes includes electrostatic, charge transfer interaction, hydrogen bonding, and modulation of hybridization [6]. This has advantages because flavoenzymes being catalysts act as the regio-, chemo- and stereo-selectivity as compared to chemical one which also helps in formation of chiral molecules in optically active form. Specially, flavoprotein oxidases and monooxygenases have a great importance in this. Moreover, they can also be used for ox functionalization [6]. This is also influenced by factors such as pH, temperature, and the presence of other molecules. It has recently discovered flavin-N5-peroxide, as a nucleophile for catalysis instead of lavin-C4a-(hydro) peroxide which expands its catalytic activity of monooxygenases [15,16].

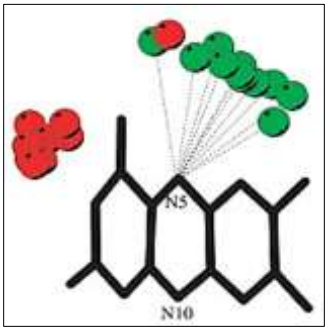

Figure 6 An overview of chemical activity of the respective flavoenzymes is related to the redox state of flavins and related reaction catalyzed. With redox states: oxidized flavin (Flox); Flavox, fully oxidized flavin, 2 electron-reduced flavin, PrFlav, prenylated FMN. The red color indicates the reactive part of the flavin

The active site of flavoenzymes has such an arrangement, relying on the nature of cosubstrate, have the ability to react with the radical equivalent, substrate carbanion equivalents and hydride equivalents and sometimes covalent adducts formation also take place between the isoalloxazine ring and substrate at different sites [8]. It is also important to be noted that isoalloxazine can adopt non-planar conformations, like polyamine and cholesterol oxidase depart from exact planarity, explained in figure 7 [11].

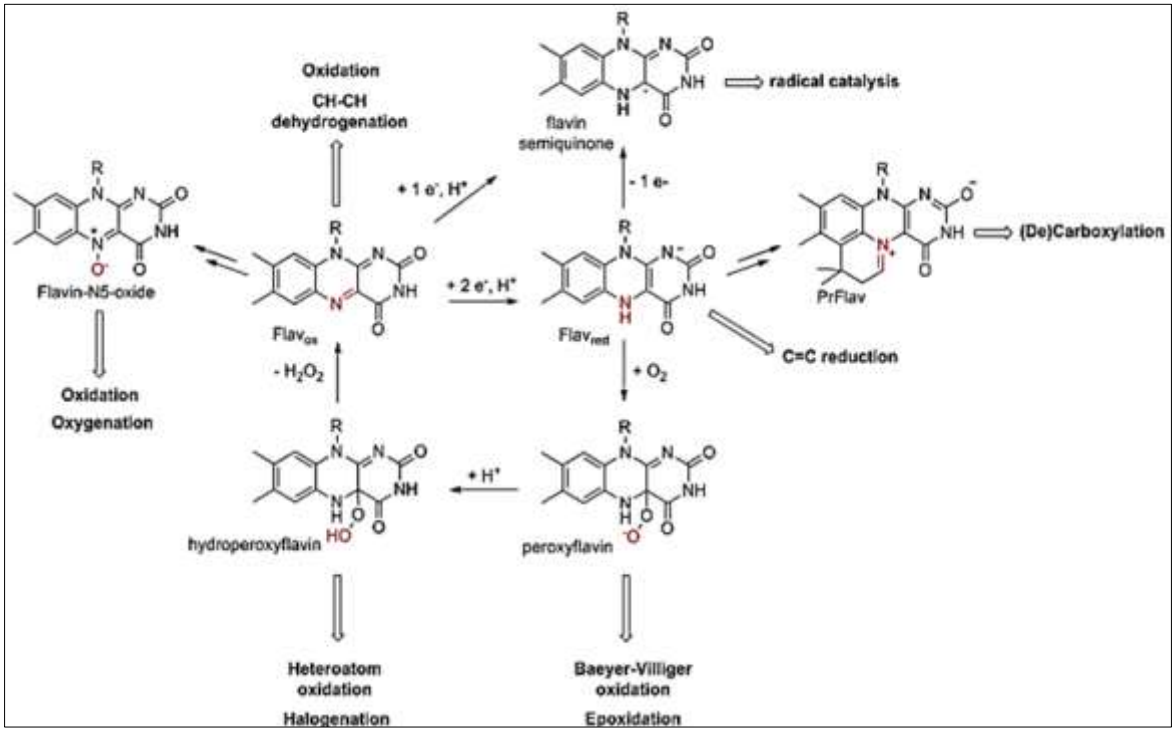

Figure 7 Flavoenzyme with Flavin-N5–protein and the substrate interaction which is stereochemical highly conserved. The hydrogen-bond donor is situated on the flavin side opposite to that facing the substrate, between N10, N5, and the hydrogen-bond donor ranges from 116^0 to 170^0. The isoalloxazine rings is for trimethylamine dehydrogenase, polyamine oxidase and cholesterol oxidase for their respective site. Green represents the protein atoms engaged in a hydrogen bond with the N5 atom while red represents carbon atoms oxidative attack site

Flavoenzymes regulate the reactivity that of an "activating group" as in case of dehydrogenation reaction by the flavoenzymes [11]. The substrate and the residues directly involved in catalysis are invariably shielded from the solvent, implying that catalysis takes place in a protected and highly controlled environment. So, enzymes mode of action depends on the precise settlement of the substrate, its modification and cofactor reactivity. Moreover, flavoenzymes can be regulated by a variety of factors, including the concentration of substrates and cofactors, the presence of inhibitors or activators, and post-translational synthesis of dopamine in neurons and dopamine metabolism by MAO-A and MAO-B [17]. The formation of dopamine intitates from tyrosine by various enzymes successively modifications [11].

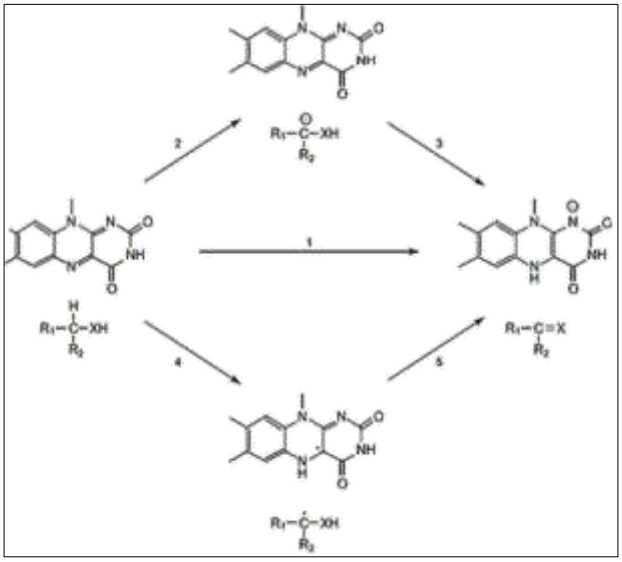

Figure 8 Mechanism for dehydrogenation of substrate using Flavoenzymes-showing three theories: Protein and flavin interactions are operative in influencing the redox properties of the cofactor. The ability of flavin to have two-electron oxidation of the substrate influenced by the hydrogen-bond interactions at the N1 and N5 loci and as a result the substrate and residue undergoes catalysis by direct hydride transfer (1), the carbanion mechanism (2,3) and the radical mechanism [4,5]. Here R1 and R2 show general substituents of the CH atom undergoing oxidation, and X is the activating group.

Enereductases of old yellow enzyme have also participated in acid-base catalysis flavin-independent. It is also important to be noted that one-electron reactivity of both the FlH_2 and FlH oxidation states allow them to react with the O_2. So, for the reaction which produces H_2O_2 the inactivation or sometimes degradation of the product can take place which can be avoided by the addition of catalase. This has also found to be reduced by morpholine-based buffers, as 3-(*N*-morpholino) propanesulfonic acid (MOPS) [18]. Moreover, they also have property of accepting and donating photons. With new flavin modification, new enzymatic synthetic system can be developed which will be useful in biotechnological and pharmaceutical area [16].

1.4 Applications

1.4.1 Flavoenzymes in Diseases

Disease detection

In Mycobacterium tuberculosis (MTB), there is an enzyme decaprenylphosphoryl-b-ribose 20 -epimerase (DprE1) which have a crucial role in the pathogenesis of the bacterium. The variant strains have led to great threat to the world. In addition, neurodegenerative disorders, detoxification, and nutrition in microbes and humans are all affected by the oxidation D-amino acids [19]**.** However, this site is the protentional target for drug synthesis and the disease can be cured. Various protentional DprE1 inhibitors have been discovered, covalently or noncovalently that can be docked and further research to target the site in future [20]. Recently, deazaflavin oxidoreductases also have found for role in treating dormant tuberculosis [21].

Neurological disorders indication

Monoamine oxidases A and B (MAO A and B) are flavoenzymes, discovered about 100 years ago, present in the outer mitochondrial membrane of mammals. This flavoenzyme has also produced in *Pichia pastoris* as a recombinant protein. They catalyze the oxidative deamination of many neurotransmitters like dopamine, serotonin and other amines which produces peroxides, and aldehydes like by-products [22]. The varied-concentration by MAO of the neurotransmitter in the brain is indication of the neurological disorders like Alzheimer's disease and Parkinson's disease (PD).

Recently, the inhibitors of the MAO [rasagiline and safinamide] and DAAO, are found to serve as the treatment of Parkinson's disease in future [23]. Because these enzymes produce less oxidative stress, this inhibition has been demonstrated to have a broader neuroprotective, neuropsychiatric and other neurological disorders impact as the oxidative stress is prevented by MAO enzymes. The activity of MAO also results in the amyloid beta (Ab) and

neurofibrillary tangles aggregation, destroying the cognitive destruction. However, the active site of MAO A and MAO B is a hydrophobic cavity that is lined with residues that are largely the same between the two isozymes, with a few minor differences that affect inhibitor and substrate specificity [22].

1.5 Drug targets and antibiotics development

NRH: quinone oxidoreductase 2 is also one of the flavoenzyme that catalyzes the reduction of quinones and pseudoquinones present in the in the cytosolic enzymes of mammals. Both NRH: quinone oxidoreductase 2 and NADH: quinone oxidoreductase 1 (NQO1) involved in quinone metabolism with later having toxifying function related to the ortho-quinones. It exhibits futile cycle and NRH: quinone oxidoreductase 2 is the target for number of drugs and natural compounds as imiquimod, melatonin, and other flavonoids. However, contempting these enzymes group's role in diseases and the functional role of NQO2 which can be overcome by the with better understanding in the future [22].

Similarly, the discovery of, acyl-CoA dehydrogenase-like flavoenzyme TdaE and its part in formation of bacterial tropone has potential applications in drug development and the development of new antibiotics. Knowing the catalytic mechanism and substrate specificity will help in not only optimizing the current drug but also for new drug formation. It has also role in the advancement of new algaecides or quorum sensing inhibitors [24].

Flavoprotein oxidases are ubiquitous enzymes involved in the biosynthesis and biodegradation of a huge variety of compounds. D-amino acid oxidase (DAAO; EC 1.4.3.3) is the proto type amino acid oxidase. This peroxisomal enzyme spreads from yeasts to humans. Mammalian DAAO has been connected to the brain D-serine metabolism and to the regulation of glutamatergic neurotransmission [20]. The outer mitochondrial membrane monoamine oxidase (MAO; EC 1.4.3.4) is perhaps the most well-known amine oxidase. In addition to the oxidation of neurotransmitters, such as dopamine and serotonin, this enzyme also oxidizes ingested amines such as phenethylamine and tyramine to prevent their functioning as false neurotransmitters. The human isoforms MAO-A and MAO-B are involved in many diseases and are important targets for antidepressant and neuroprotective drugs. Acyl-CoA oxidases (EC 1.3.3.6) are acyl-CoA dehydrogenase homologs involved in peroxisomal fatty acid breakdown in plants in addition to the oxidation of neurotransmitters, such as dopamine and serotonin, this enzyme also oxidizes ingested amines such as phenethylamine and tyramine to prevent their functioning as false neurotransmitters. The human isoforms MAO-A and MAO-B are involved in many diseases and are important targets for antidepressant and neuroprotective drugs [22]. Acyl-CoA oxidases (EC 1.3.3.6) are acyl-CoA dehydrogenase homologs involved in peroxisomal fatty acid breakdown in plants.

1.6 Novel drugs and therapeutic approaches

Aldehyde Oxidases (AOXs) enzymes are small sub-family of cytosolic molybdoflavoenzymes, which are widely distributed in plants and animals. They play an important role in both physical and pathological process in the body and its inhibition is used for the formation of novel drugs and therapeutic approaches as for cancer, aging, obesity and amyotrophic lateral sclerosis. Raloxifene, AOX inhibitor, used for reducing the oxidative stress in human placental cells but also accepted for the breast cancer reduction in postmenopausal women. Moreover, this enzyme also inhibits the metalloproteinase-2 (MMP-2) enzyme which is the cause for tumor invasion and angiogenesis beginning in breast cancer. So, the inhibition of AOX1 may avert oxygen radical metabolism and help in relieving ALS and other associated therapeutic strategies. Indeed, AOX polymorphisms in disease being explained, new therapeutic approaches will be gained. Inhibition of vertebrate aldehyde oxidase as a therapeutic treatment for cancer, obesity, aging and amyotrophic lateral sclerosis [25,26]. D-amino acid oxidase [DAAO] can be used in various applications including determining D-amino acids and creating a substance for semi-synthetic cephalosporins [27].

1.7 Disease treatment

Ferroptosis is the iron-dependent cell death determined by the peroxidation of membrane lipids. This is intricated in pathogenesis of a number of various diseases. This property can also be used to kill cancerous cells as a new therapeutic approach. And number of catalytic mechanisms of ferroptosis involved the flavoenzymes, are beneficial to treat neurodegeneration and stroke like issues by comprehensive understanding of human-flavoproteome in proteome. The balance between the pro and anti-ferroptotic roles played by flavoproteins, as well as how this equilibrium can be changed in either way depending on therapeutic requirements, are the main questions that need to be clarified in the future [28].

Another target for cancer therapy is a flavoenzyme called proline dehydrogenase (PRODH) catalysis, the first stage of proline catabolism. Recently, two ,3-dithiolane-2-carboxylate and tetrahydrothiophene-2-carboxylate have found to role in the metabolic reprogramming of cancer cells which provides the drug target for this [29]. Riboflavin has very important role in maintenance of human health like stress responses, mitochondrial function stability and flavoenzyme

stability. However, its lack in diet result in sever complication especially when combined with inborn genetic problems and physiological factors (infections, aging, diet), other environmental. So, this source has reported for riboflavin treatment to cure various diseases related to this as mentioned [30].

1.8 Biotechnological applications

From the recent crucial investigation and implication from number of organisms about D-amino acids has tremendously increased its importance. It is present from animals to mammals. DDO may also be a useful tool in a number of biotechnological applications, including the identification and measurement of acidic D-amino acids to investigate the physiological roles played by them. It has also been recent research that a flavoenzyme has discovered that oxidize the plant cell-wall. They have countless importance in biology and biotechnological applications [31]. Cholesterol oxidase, another flavoenzyme also has role in industry, insecticidal property and steroid drug property [32].

In biotechnology, the enzymatic synthesis of 2,5-furandicarboxylic acid (FDCA) is crucial. The selection and development of HMFO enzymes as biocatalysts for the enzymatic synthesis of renewable building blocks in the manufacture of bioplastics can be facilitated by gene screening and heterologous expression [33].

D-amino acids are used at the host-microbe interface as a means of inter-kingdom communication to control bacterial colonization and host defense. There are many different types of D-amino acids produced by the microbes as compared to eukaryotes and archaea which are used as the antimicrobial activity. This is because of D-amino acids oxidation that results in H_2O_2 production indicating the response in mammals. The growth of some bacteria that are dependent on host nutrition is modulated by intestinal DAO, which also changes the microbiota's makeup. Additionally, D-serine has bacteriostatic properties in the urinary tract, D-leucine inhibits innate immunity through the sweet taste receptor in the upper airway, D-phenylalanine and D-leucine modulate immune tolerance in the lower airway, and D-tryptophan regulates neutrophil chemotaxis through a G-coupled protein receptor [34].

DAO has dual effects on the luminal microorganisms in the gut mucosa. It also alters the availability of D-amino acids for bacterial growth, which in turn influences the composition of the commensal microbiota. On the other side, by producing H_2O_2 through the oxidation of bacteria [34].

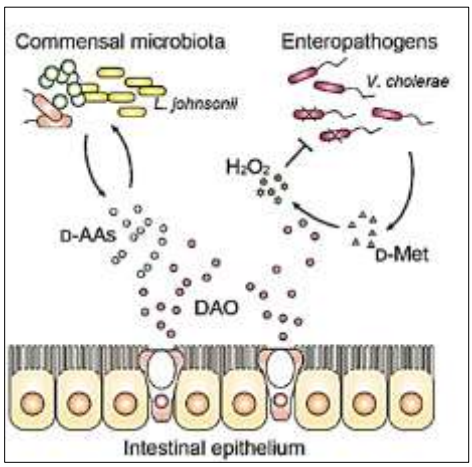

Figure 9 D-amino acids, DAO restricts the colonization of enteropathogens like *Vibrio cholerae.*

1.9 Role of flavoenzymes in various industries

Sugar oxidases are group of flavoenzymes that convert the carbohydrates which theoretically transform the sugars to produce the beneficial products not only for other sugars but also for fine chemicals and drugs. Indeed, knowledge on the connection between the structure and function of these sugar oxidases highlights the essential characteristics of this particular set of engineering-modifiable enzymes, which have led to an exceptional economic importance [35]. It is crucial for the chiral building blocks that are made for the pharmaceutical and food industries as well as the resolution of racemic amino acid combinations. Recent years have seen a significant accumulation of biochemical and structural understanding regarding the enzymes responsible for oxidizing the C-N bond of D-amino acids. With new structure developments of these enzymes the new applications will also develop in near future [19].

Beside that alcohol oxidase has number of applications including biocatalytic formation of many carbonyl compounds use in flavor, pharmaceutical and clinical industries [36]. Infact, modified D-amino acid oxidases have produced to enable them to use as biocatalyst for determing racemic amino acid mixtures, as herbicide resistant and biosensor [37].

Vanillyl alcohol oxidase (VAO) is a fungal flavoenzyme and subgroup of the VAO/ PCMH flavoprotein family. It is involved in the conversion of various para-substituted phenols into coniferyl alcohol, vanillin and chiral aryl alcohols. They are divided into three clades, two of which missing defined members. These products have great importance in the number of industries. However, there is very little information about the physiological role and its distribution [38]. Another important application is their use as for the production of high optically pure acidic L-amino acids, α-amino acids, and non-natural amino acids. These compounds are used as precursors in pharmaceutical and chemical industries which are produced by coupling of DDO with non-selective reagents as amine-boranes, however, some factors need to be considered [39].

1.10 Environmental applications

In the environment, there is accumulation of halogen-containing due to their use in many industries produced or naturally by marine organisms. This is due to lack of degradation biological pathway unless the flavoenzymes (deiodinases and thioredoxin-like reductive debrominase, Bmp8) have discovered their role in degradation with C4a-hydroperoxyflavin and covalent intermediate using oxidative, reductive and hydrolytic pathways. This will greatly save the environment to reduce the pollutants in future by use of engineering techniques in future [40]. Similarly, nitroaromatic compounds also get degraded by various flavoenzymes by single- and two-electron reduction mechanism [41].

Moreover, due to modification of flavins molecules to enhance the catalysis, reactivity or selectivity for applications respectively. This has drastically altered to for applications in biomedicine or water remediation by flavin photocatalysts using polydopamine as a carrier. This is possible due chiral linkage between PDA and flavin, allow the product to be enantioselective [42].

Enzymes of potential use as biocatalysts in the degradation of hazardous chemicals such nitrophenols and halogenated phenols, which are frequently present in the environment, are found in *Ralstonia pickettii* DTP0602's Had operon. These enzymes may play a role in future bioremediation applications [43].

1.10.1 Biosensor

Due to the excellence in enantioselectivity and stereoselectivity, D- amino acids have number of applications in biotechnology as one of them is their use as biosensors including for analyzing bacterial contamination in foods and beverages. Fungal DDO are preferable for their beneficial in comparison to animal DDOs due to its strong catalytic activity, close cofactor binding, and greater stability [39].

Dye-linked D-amino acid dehydrogenases (Dye-DADHs) are enzymes that, in the presence of a synthetic electron acceptor, catalyze the dehydrogenation of free D-amino acids. After discovery in mesophilic gram-negative bacteria, novel dye-DADHs has identified in thermophilic bacteria and hyper thermophilic archaea, found to have application in electrochemical biosensors [44].

In addition, sugar oxidases have been used in the food industry for biosensing different biological molecules, as pyranose 2-oxidase (P_2O) and glucose oxidase (GO), disease assessment, and environmental contaminant detection. Alcohol oxidases are also used for biosensor development. Now, third generation biosensors formation is also possible whereas further research is going on [36].

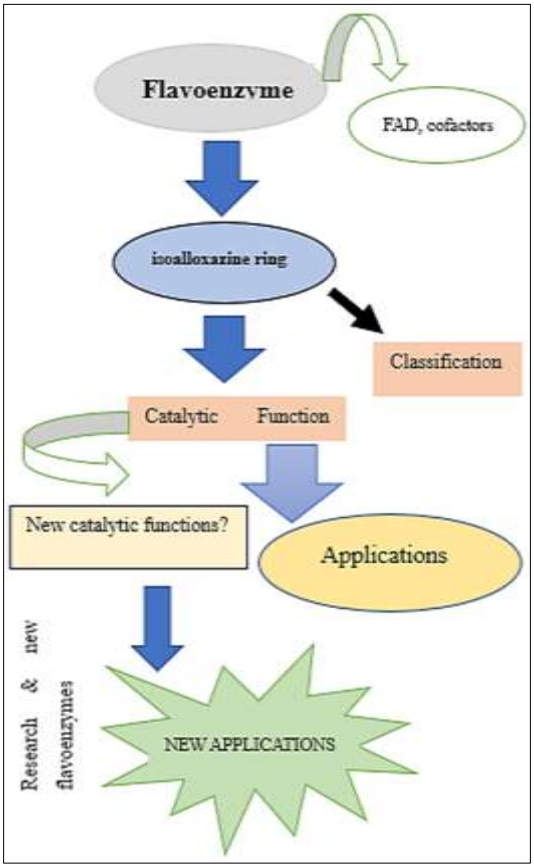

Figure 10 Flowsheet for flavoenzyme-review article

1.10.2 Some others applications

Similarly light activation of flavoenzymes led to a number of inventive synthetic applications that provided new reaction modes which addressed the previous challenges with light activation of flavoenzymes. However, this is all have limited to the CvFAP and ERED enzyme classes. In the near future, new photoenzymes may be discovered as well as more varied flavoprotein incorporations in photochemocatalytic reactions [45].

It has also been experimented that the use of flavoenzymes in photocatalytic and photoswitching processes may lead to further investigation of photoisomerization processes involving CT transitions, providing further benefits [45,46].

It is recently researched that monooxygenation of aromatic compound by flavin-dependent monooxygenases have led to the new identification of two new classes: single-component and two-component flavin dependent hydroxylases (monooxygenases). The beauty of this class is that it modifies the biological characteristics of phenolic compounds such that the new compounds are being synthesized. Enhancing enzyme engineering and redesigning, which can result in the development of biocatalytic processes for the production of valuable chemicals, requires an understanding of the catalytic residues and active site conditions that influence enzyme reactivity [47].

Flavoenzymes also have discovered to form gold nanoparticles and (AuNPs) or nanoclusters (AuNCs) are formed for optical biosensor development. This is due to oxidation of glucose by Gox enzyme happens in the presence of Au (III) which is linked to the glucose depending on reaction conditions. Furthermore, depending on the operating pH, this can be utilized to determine the substrates by fluorescence [AuNCs] or molecular absorption (AuNP). Further enzymes need to be researched for further applications [48].

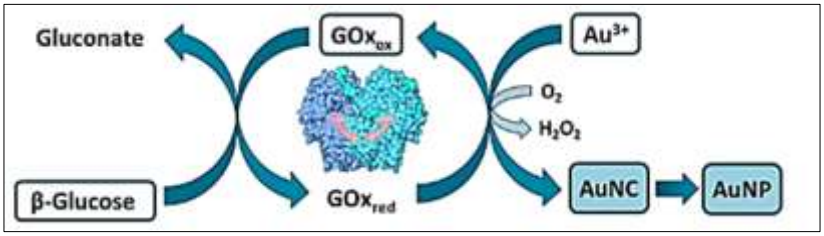

Figure 11 Glucose oxidase catalysis the enzyme-mediated oxidation of glucose in the presence of Au (III) and results in the formation of gold nanoparticles and (AuNPs) or nanoclusters (AuNCs)

Flavin ring system has been immobilized on poly (methacrylic acid), PMAAs under suitable conditions which have reaction for aerobic oxidation catalysis which have number of applications, with stereoregularity effect of polymers on enzymes [49].

The viability of employing flavoenzymes and coenzyme biomimetics for regioselective aromatic hydroxylation, an important chemical process for creating pharmaceutical molecules, has also been studied. The use of biomimetics also seem to less uncoupling of hydroxylation and hydrogen peroxide production which is a promising alternative for industrial applications. It is also important to be noted that many of the flavoenzymes have modified or coupled which have resulted in better understanding of the respective mechanism and its tremendous applications [25].

2 Conclusion

Flavoenzymes are very diverse enzymes group that play an important role in biological system. They are classified into several classes depending on the reaction they catalyzed due to isoalloxazine ring, includes hydroxylation, dehydrogenation, and epoxidation, among others. These reactions have significant role in biological system and have wide applications majorly medicine, agriculture, biotechnology and industries etc. However, with the mentioned examples it can be concluded that with the better understanding of the reaction mechanism of various flavoenzymes classes, more tremendous applications will be explored. Identification of novel flavoenzymes especially photoenzymes can be expanded and new applications can be made. Uptill now many of the flavoenzymes have coupled for better understanding of the catalytic mechanism and discovered gold nanoparticles and (AuNPs) or nanoclusters (AuNCs) having role in optical biosensor development. Infact, the research is going on and further study is required to fully investigate flavoenzymes potential in various fields and their synthetic applications.

Compliance with ethical standards

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Disclosure of Conflict of interest

There is no such conflict of interest.

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