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CHEMICAL APPLICATIONS OF CLASS B FLAVOPROTEIN MONOOXYGENASES

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Abstract

Biocatalysis using flavoprotein monooxygenases is coming of age, with intense research and development being carried out within the last decade. The reason behind their popularity is the vast array of reactions which they can catalyze including Baeyer-Villiger oxidation, sulfoxidation and epoxidation reactions. Members of Class B flavoprotein monooxygenases, especially Baeyer-Villiger enzymes, are highly selective in their chemo-, regio-, and enantioselective oxygenation reactions and are useful in the synthesis of high-value chemicals. Their catalysis products have wide applications in various fields including chemical, cosmetic as well as pharmaceutical industries. Moreover, in the era of a drive for more environmentally-friendly reactions with the use of less toxic reagents and ambient temperatures, these flavoproteins are well-suited to the principles of green chemistry.

This mini review provides an overview of some stereoselective reactions carried out by Class B flavoprotein monooxygenases and the efforts made in order to make these biocatalysts suitable for industrial applications.

Keywords: Baeyer-Villiger, flavin-containing monooxygenase, biocatalysis, sulfoxidation, stereoselective, drug metabolite, industrial application

1. INTRODUCTION

Flavoprotein monooxygenases are a large class of enzymes that are responsible for the activation and utilization of molecular oxygen for the monooxygenation of a many different compounds including drugs, pesticides and other xenobiotics. During their catalytic cycle, one atom of molecular oxygen is incorporated into their substrate, while the other is reduced to water. The key step in their catalytic cycle is the formation of a flavin intermediate, C4a-oxygen adduct (Massey, 1994). Only the reduced flavin can react and activate molecular oxygen. The monooxygenase systems are typically reduced by a physiological electron partner, either NADPH or NADH, that donates the electrons required for catalysis. Since the electron source is not the substrate itself, they are called “external monooxygenases”. It is believed that the reduced flavin upon binding oxygen, generates a radical pair between the one electron reduced flavin and the superoxide radical (Massey, 1994). The latter radical pair gives rise to the C4a-(hydro)peroxyflavin intermediate, that can oxygenate the substrate. The C4a-(hydro)peroxy intermediate of each monooxygenase system can decay with a different velocity, whilst a nucleophilic or electrophilic attack on the substrate will take place depending on the protonation state of the flavin. Several crystal structures of flavoprotein monooxygenases are now available (Malito et al., 2004; Eswaramoorthy et al., 2006; Alfieri et al., 2008; Mirza et al., 2009) and they can be used together with sequence alignment to predict and understand folding determinants that are responsible for carrying out monooxygenation reactions (Gao et al., 2016). Furthermore, these crystal structures also show the relatively large and accessible active sites of these proteins which could be one reason for their promiscuity (Figure 1).

2. CLASSIFICATION

Flavoprotein monooxygenases can be classified using different parameters such as the reaction that they catalyze, the type of substrates they can accommodate and/or their conserved structural elements. However, in many cases the crystal structure of the members of this vast group of enzymes is not available, so the classification is mainly based on sequence homology rather than structural data. Van Berkel and colleagues (Van Berkel et al., 2006) proposed six different groups (A-F) based on structural and functional properties of flavin-dependent monooxygenases.

Class A monooxygenases are encoded by a single gene, contain a tightly bound FAD cofactor, can work with NADPH or NADH, they release NADP⁺ immediately upon flavin reduction,

they contain one nucleotide binding domain for FAD. The C4-hydroperoxyflavin performs electrophilic attack on the aromatic ring of an activated compound containing an activated nitrogen or hydroxyl group (Moonen et al., 2002).

Class B monooxygenases are also encoded by a single gene, contain a tightly bound FAD cofactor, depend on NADPH as coenzyme, keep the NADP⁺ bound during catalysis and are composed of two dinucleotide binding domains (for FAD and NADPH). Enzymes belonging to this class can catalyze carbon, nitrogen, sulfur or phosphorous oxidation. Three subfamilies can be identified within this class on the basis of their sequence features (Fraaije et al., 2002): flavin-containing monooxygenases (FMOs), microbial N-hydroxylating monooxygenases (NMOs) and Baeyer-Villiger monooxygenases (Type I BVMO).

Unlike class A and B, Class C monooxygenases are encoded by multiple genes encoding for one or two monooxygenase components and a reductase component, use FMN as coenzyme and can use NADPH or NADH (Fischer et al., 1995). Bacterial luciferases and Type II BVMOs are the most studied examples of enzymes that belong to this class.

Classes D-F are encoded by two genes, a monooxygenase and a reductase, use FAD as a coenzyme and can use either NADPH or NADH as their electron donor.

In this mini review we will focus mainly on the description of two subfamilies that belong to Class B: Baeyer-Villiger monooxygenases (BVMO) and flavin-containing monooxygenases (FMO).

3. BVMO

The chemical Baeyer-Villiger reaction was discovered in 1899 by Adolf von Baeyer and Victor Villiger (Baeyer and Villiger, 1899). In this oxidation mechanism a ketone compound is attacked by a nucleophilic peroxy acid to form a tetrahedral intermediate called “Criegee intermediate”. This species is unstable and undergoes a rearrangement through an expulsion of a carboxylate ion and a migration of a carbon-carbon bond, resulting in an ester or lactone being formed and a carboxylic acid side product (Ballou and Entsch, 2013). The obvious disadvantages of the chemical reaction are both the need for a peracid as reagent and the equimolar production of an undesired carboxylic acid. These chemical oxidants utilized as reagents are not only expensive, but tend to be unstable and even explosive, presenting a safety risk. Moreover, the side product of the reaction (a carboxylic acid that depends on the organic peracid used as oxidant) must be recycled or disposed.

Baeyer-Villiger monooxygenases (BVMOs) catalyze specific Baeyer-Villiger oxidations on the carbonyl moiety of substrates with high regio- and enantioselectivity. In addition, they can

also catalyze the electrophilic heteroatom oxygenation including nitrogen and sulfur (Walsh and Chen, 1988). These proteins have a great value in the conversion of ketones into the corresponding esters and lactones, bioremediation purposes and green chemistry applications such as the synthesis of chiral intermediates. Unlike the chemically driven B-V reaction, the BVMOs carry out their catalysis without the use of strong chemical oxidants and without formation of side products, but more importantly as mentioned above, in a highly regio- and enantioselective manner.

BVMO enzymes are abundant in bacterial, fungal and plant genomes but nearly absent in animal or human genomes. They are involved in metabolic pathways (Chen et al., 1988; Sheng et al., 2001; Minerdi et al., 2015) and in some cases have medical relevance (Fraaije et al., 2004; Minerdi et al., 2012; Catucci et al., 2016; Minerdi et al., 2016). The first BVMO enzyme that was isolated and purified was a cyclohexanone monooxygenase (CHMO) from *Acinetobacter* NCIMB 9871 (Donoghue et al., 1975). Since the late 90s many other BVMO enzymes have been cloned and characterized in terms of catalytic activity towards a broad range of compounds. A huge leap forward in the understanding of these enzymes was subsequently achieved with the publication of the first crystal structure of a type 1 BVMO, Phenylacetone monooxygenase (PAMO) (Malito et al., 2004).

All BVMO enzymes characterized to date catalyze a monooxygenation incorporating one atom of molecular oxygen into the substrate and reducing the other to water (Figure 2). The catalytic mechanism is similar to those previously described in literature for other flavin-containing enzymes with the addition of the Criegee intermediate.

3.1. BIOCATALYSIS by BVMOs

BVMOs present various catalytic activities and a wide substrate range, making them perfect as biocatalysts for large-scale synthetic applications as well as biotechnological applications. The first BVMO to be studied in detail was cyclohexanone monooxygenase (CHMO) from *Acinetobacter sp.* (NCIMB 9871) which converts a cyclic ketone to a lactone, which can subsequently be hydrolyzed to an aliphatic acid. Under physiological conditions, CHMO catalyzes a key step in the biodegradation of cyclohexanol (Sheng et al., 2001). These enzymes are present in different bacterial genomes and have been studied in depth for many years.

Acinetobacter calcoaceticus NCIB 9871 is a bacterial strain also capable of growing on cyclohexanol as its sole carbon source (Donoghue et al., 1975). The metabolic pathway for the degradation of the alcohol involves the CHMO in the second step of the oxidation. Adipic acid, as a final product of this metabolism, is then further oxidized during β -oxidation to form acetyl-

CoA and Succinyl-CoA. This pathway can be exploited both for the synthesis of ϵ -caprolactone and adipic acid. In the former case, the CHMO product can be used to produce polycaprolactone via ring opening polymerization (Labet et al., 2009). Polycaprolactone (PCL) is a polyester with interesting physical and chemical properties and, more importantly, it is also biodegradable. Whereas, in the latter case, industrial production of adipic acid could be applied to Nylon 6,6 synthesis (Leisch et al., 2011). Nylon 6,6 is a polymer obtained from the condensation of adipic acid and hexamethylenediamine to form a 12 carbons repeating unit. Currently the synthesis of these compounds is still performed chemically (Raja et al., 2006) but in the near future biocatalysis could be an interesting alternative to this approach.

CHMO from *Acinetobacter calcoaceticus* NCIMB is another well-studied of these CHMO enzymes. In 1988 Stewart published a paper in which he used this CHMO for the synthesis of (bicyclic) lactones (Stewart, 1988). Colonna and co-workers in 1996 showed that the same CHMO has a wide substrate selectivity towards organic sulfur compounds. They were interested in synthesis of various sulfoxides not only due to their involvement in many biological activities but also for their pharmaceutical importance. They demonstrated that this enzyme was able to oxidize alkyl aryl sulfides, disulfides, dialkyl sulfides, cyclic and acyclic 1,3-di- thioacetals and 1,3-oxathioacetals to the corresponding mono-sulfoxides, with high e.e.s (Colonna et al., 1996). A couple of years later, the same research group showed that CHMO was also able to oxidize organic cyclic sulfites to their corresponding sulfates (Colonna et al., 1998). Cyclic sulfites and sulfates are considered to be the synthetic equivalents of epoxides, capable of reacting with a large variety of nucleophiles.

To date, more than hundred different substrates for CHMO have been identified and the reader is referred to a detailed review by Mihovilovic's group (Mihovilovic et al., 2002).

Another interesting BVMO, this time active on aromatic compounds, is 4-hydroxyacetophenone monooxygenase (HAPMO) which was isolated from *Pseudomonas fluorescens* ACB (Higson and Focht, 1990). Substrate specificity of this enzyme covers different ketones including aryl ketones. Conversion of ring-substituted aryl ketones into phenyl acetates by HAPMO provides an alternative route for synthesis of precursors of pharmaceutically important compounds (Held et al., 1999). Fraaije's group have published a review including the many different reactions catalyzed by HAPMO (Kamerbeek et al., 2003).

BVMOs have another interesting characteristic that can be exploited for organic synthesis. These enzymes perform highly regio- and enantio- selective reactions on both ketone and soft nucleophilic substrates. In the chemical Baeyer-Villiger oxidation the oxygen insertion occurs

only within the carbonyl carbon and the most substituted adjacent carbon of the substrate whereas, interestingly, BVMOs show regiodivergency on some substrates. This means that according to the enantiomeric properties of the substrate, different regioisomers of the product can be obtained. An interesting example is the regiodivergent conversion of (\pm)Bicyclo[3.2.0]hept-2-en-6-one in the two corresponding regioisomer products: (-)-(1S, 5R)-2-oxabicyclo[3.3.0]oct-6-en-3-one and (-)-(1R, 5S)-3-oxabicyclo[3.3.0]oct-6-en-2-one. This reaction is also highly enantioselective yielding nearly 1:1 ratio of the regio-isomer products with high enantiomeric purity (Hilker et al., 2008). This “unexpected” lactone can be used as precursor for the synthesis of several brown algae pheromones such as multifidene and viridene (Lebreton et al., 1997).

Another BVMO, cyclopentadecanone monooxygenase (CPDMO) from *Pseudomonas* sp. strain HI-70, was shown to prefer cyclic and bicyclic ketones with 7–16 carbons atoms (Iwaki et al., 2006). When tested with 33 ketosteroids, another group (Beneventi et al., 2009) showed that CPDMO is not only able to accommodate in its active site flexible rings such as cyclopentadecanone, but also more structurally demanding compounds such as steroids. The enzyme was able to catalyze the B-V oxidation of 3-keto and 17-ketosteroids with full control of the regiochemistry of the produced lactones (Beneventi et al., 2009). Steroids are a highly sought after class of natural products with a multitude of pharmacological properties.

BVMOs are often reported in literature as performing the resolution of racemic mixture of substrates. An interesting study on both PAMO and HAPMO reports this catalytic activity towards several substituted benzylketones (Rodriguez et al., 2009). Both PAMO and HAPMO can perform highly enantio-selective resolutions of racemic ketones presenting a good method for the preparation of both enantiomerically pure benzyl ketones and esters.

Finally, some of the different reactions carried out by Class I BVMO enzymes are summarized in Table 1.

3.2. INDUSTRIAL APPLICATION

Nowadays enzyme cloning, expression and purification has become routine and there are many variety of ways to produce the BVMO biocatalysts that can be exploited for the generation of the desired chemical product. However, the main bottleneck in using BVMOs in large scale chemical production, is their dependence on stoichiometric amounts of the reduced nicotinamide coenzyme NADPH (Torres Pazmino et al., 2010) and its high cost. There are several solutions to overcome this problem, some of which are based on the recycling of the

oxidized cofactor that is the by-product of the enzymatic reaction. In order to do so, one needs to find a way to catalyze the reverse reaction yielding the reduced form of NAD(P) that is needed for the reaction of interest. The approach can either be chemical, electrochemical, photochemical or enzymatic.

3.2.a COFACTOR RECYCLING

In the chemical approach, regeneration of the nicotinamide coenzyme can be performed by exploiting rhodium complex $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})]^{2+}$ (Hollmann et al., 2002). The use of this compound leads to the formation of CO_2 which can be easily removed from the reaction. Unfortunately, this chemical reaction occurs under alkaline conditions and it is hardly compatible with many enzymatic reactions (Hollmann and Schmid, 2004).

Another tested method is electrochemistry. The main advantage of using electrochemistry for cofactor regeneration is due to the low cost and the ease of separation of the products. The regeneration can be a direct reduction occurring at the cathode electrode (Hollmann et al., 2001; Kim and Yoo, 2009). The use of mediator can be exploited for indirect reduction of the coenzymes (Sadeghi et al., 1997; Hollmann et al., 2001). The mediator can also be a protein that shuttles the electrons from the cathode to the cofactor selectively (ferredoxin NADP^+ reductase, NAD(P)^+ dehydrogenases). Immobilization techniques on the electrode surface are also available and can be used for cofactor regeneration or bypassing the need for NADPH (Castrignano' et al., 2010; Sadeghi et al., 2010; Castrignano' et al., 2012; Castrignano' et al., 2015).

Another attractive method is the photochemical approach where solar light can be used to reduce light-sensitive material that can be exploited for the electron transfer following the absorption of light. Examples of these light-sensitive materials include $[\text{Ru}(\text{bpy})_3]^{2+}$, Zn-TMPyP^{4+} , flavin dyes, Fe_2O_3 and TiO_2 that are able to generate electrons from electron donors such as EDTA. The reduction occurs through an electron carrier, like methyl viologen, that is able to transfer the electrons to NAD(P)^+ -dependent enzymes. This approach was successfully demonstrated by Reetz's group showing the BVMO oxidation of prochiral ketones (Hollmann et al., 2007). However, the poor efficiency of this system needs to be addressed.

The enzymatic regeneration of the nicotinamide cofactor seems to be the method of choice with more publications than any other regeneration system. The coenzyme regeneration systems are based on a coupled enzymatic reaction that produces NADPH at the expense of another substrate. The sacrificial substrate is an important point to consider as it can influence the cost of the reaction. Typical coupled enzymes include glucose-6-phosphate dehydrogenase,

alcohol dehydrogenase and glucose dehydrogenase. A more recent alternative has been the production of fusions using protein engineering between these enzymes and BVMOs in order to produce a self-sufficient BVMO (Torres Pazmino et al., 2009; Ceccoli et al., 2014).

3.2.b WHOLE CELL

A more comprehensive approach that allows both desired product formation and cofactor regeneration is the use of whole cell systems since the host cell provides the NADPH. In this type of set up the biocatalyst is expressed by the host cell either by genetic engineering of the host, typically *E. coli* or yeast, or transforming the host with a plasmid containing the coding sequence of the desired target enzyme. The clone expansion into a suitable media for growth via fermentation will result in the production of microbial cells containing the desired catalysts ready to perform the enzymatic reaction. During the fermentation the microbial cells are supplemented with the substrate of interest that is converted by the enzyme. At the end of the process the product is extracted using an organic solvent separation methodology.

There are a number of examples of successful whole-cell catalysis using the BVMO enzymes. In 2005, Rial et al. used the whole-cell approach to perform a screening on the transformation capabilities of *Xanthobacter sp. ZL5*. In their work Rial and colleagues performed a substrate profiling using a collection of mono-substituted cyclic ketones, fused bicyclobutanones and terpenone derivatives (Rial et al., 2008). Dudek and colleagues in 2013 developed a generic whole based method for BVMOs (Dudek et al., 2013). The latter research group used the PAMO from *Thermobifida Fusca* and decided to direct the functional protein to the periplasm, so that the substrate could always be available to the enzyme and to enhance the exchange of the cofactor with the phosphite dehydrogenase (PTDH) which was the cofactor regenerator. Interestingly the byproduct of PTDH, phosphate, can also be used to assess BVMO assay (Dudek et al., 2013). The molybdate assay, through colorimetric detection, was employed to quantify phosphate.

More recently Summers and co-workers identified a BVMO in *R. jostii* that catalyzed the regioselective biotransformation of bicyclo[3.2.0]hept-2-en-6-one to the corresponding lactone. Also in this case the enzyme, named MO14, was tested in a whole-cell fermentation system investigating the biotransformations on a gram scale (Summers et al., 2015). The whole-cell system offers economical advantages over purified enzymes, but also poses more problems in terms of reproducibility of the results and production of unwanted byproducts. Moreover, the substrate cannot always enter the cell membrane and the product once formed could also be degraded due to the metabolism of the host.

Pure enzyme-based catalysis remains a valuable way to produce high added value products and whole-cell systems can overcome some of the economical barriers that prevent some enzymes and specifically monooxygenase systems from being industrially exploited.

4. Flavin-containing monooxygenases (FMOs)

Apart from class I BVMOs, another group of enzymes called flavin-containing monooxygenases (FMOs) are also part of Class B flavin-dependent monooxygenases (Van Berkel et al., 2006). Unlike BVMOs, the FMOs are also present in animals and humans. The mammalian FMOs comprise a family of five functional enzymes (FMO 1-5) and are membrane-bound, generally making them more difficult for use in biocatalysis. However, a more soluble version of the human FMO3 enzyme has been reported by the truncation of the membrane-anchor (Catucci et al., 2012). In general, the FMO enzymes catalyze the oxygenation of a wide variety of nucleophilic heteroatom-containing compounds, mainly sulfur and nitrogen (Figure 3). The mammalian FMOs are classified as drug metabolizing enzymes and show overlapping substrate spectra with cytochromes P450.

The FMO-catalyzed oxygenation reaction can be divided into two sequential reactions, the reductive and oxidative half-reactions. In the reductive half-reaction, the FAD cofactor is first reduced to FADH₂ by NADPH and subsequently reacts with molecular oxygen to yield the C4a-(hydro)peroxyFAD intermediate. During the oxidative half-reaction and in the presence of substrate, an oxygen atom is transferred from the intermediate to a substrate to generate an oxygenated product and H₂O. Structures of protein-cofactor and protein-substrate complexes for a eukaryotic FMO (yFMO) from *Schizosaccharomyces pombe* (Eswaramoorthy et al., 2006) and a prokaryotic FMO (mFMO) from *Methylophaga sp.* Strain SK1 (Alfieri et al., 2008) provide insights into their possible mechanisms of action.

The best studied bacterial FMO, mFMO, is active against N- and S-containing compounds such as trimethylamine and thiourea but its notable reaction is its ability to produce indigo blue (Choi et al., 2003). More recently, a fusion of this enzyme and PTDH has been reported (Rioz-Martinez et al., 2011). PTDH-mFMO was tested by these authors for enantioselective oxidation of thioanisole derivatives, with the most effective conversion yielding 95% of (*S*)-methyl phenyl sulfoxide. The fusion enzyme was also shown to oxidize methyl *p*-tolyl sulfide and *p*-chlorophenyl methyl sulfide into the corresponding sulfoxides with high enantiomeric excesses i.e. more than 90%. These authors also successfully demonstrated the oxidization of indole and analogues into the corresponding indigoid pigments by PTDH-mFMO (Rioz-Martinez et al., 2011). The latter pigments are interesting dyes and bioactive compounds. In 2012, work on a

first marine bacterial FMO was published (Jensen et al., 2012) which was shown to catalyze both B-V oxidations of carbonyl compounds as well as sulfoxidation of prochiral sulfides. An example of the latter sulfoxidation reaction was demonstrated by obtaining (*R*)-4-chlorophenyl methyl sulfoxide with an optical purity of > 80%.

Moving on from the bacterial to animal FMOs, the applications of especially mammalian FMOs in chiral separations are hampered and little-studied due to their typically membrane-associated and thermal instability. However, some researchers have demonstrated the involvement of these mammalian enzymes in specific chiral reactions. For example, the purified rabbit lung FMO2 has been shown to be remarkably stereoselective for the sulfoxidation of methyl *p*-tolyl sulfide with an enantiomeric excess of 96% for the (*R*)-methyl *p*-tolyl sulfoxide (Rettie et al., 1994). Another example is that of Sulindac, a nonsteroidal anti-inflammatory drug that contains a chiral sulfoxide moiety and is clinically administered as a racemic mixture. Sulindac sulfoxide is reduced *in vivo* to sulindac sulfide (Figure 4), the pharmacologically active metabolite (Hamman et al., 2000). In serum and urine of dosed volunteers, a distinct stereoselective enrichment of the (*R*)-sulindac sulfoxide is observed. Hamman and coworkers showed that purified mini pig liver FMO1, rabbit lung FMO2, and human cDNA expressed FMO3, all efficiently oxidized sulindac sulfide with a high degree of stereoselectivity towards the *R*-isomer (Hamman et al., 2000). In case of the human liver microsomes, FMO3, the (*R*)-sulindac sulfoxide was obtained with an e.e. of 87%. FMO1 and FMO2 were even more stereoselective in the formation of (*R*)-sulindac sulfoxide, with an e.e. of 98 and 99%, respectively (Hamman et al., 2000). These data are summarized in Table 2. In other examples, albendazole and fenbendazole sulfoxides, benzimidazole derivatives with anthelmintic activity were obtained using liver microsomes from sheep and cattle (Virkel et al., 2004). The authors showed that the sulfoxidation of albendazole led to the enantiopure sulfoxide. Another research group (Furnes and Schlenk, 2004) demonstrated how their FMO1 mutants could oxidize the insecticide fenthion with excellent selectivity for the (+)-sulfoxide.

Potential application of human FMOs

In course of drug development process, drug metabolites are required for structure elucidation as well as standards used in analytical separations. Chemical synthesis of these drug metabolites requires multiple reaction steps including the protection and deprotection steps of some functional groups. However, this process can also be carried out by heterologously expressed and purified human FMOs in a single catalytic step *in vitro* (Catucci et al., 2013). As mentioned previously for the BVMO enzymes, whole-cell biocatalysis where time and costs

of enzyme purification as well as cofactor recycling are obviated, can also be applied to the FMO enzymes. To this end, a recent study using human FMO3 in a whole-cell set up was reported for the synthesis of a high-priced human drug metabolite, moclobemide-*N*-oxide, on the multi-milligram scale (Halon et al., 2012). This study not only demonstrated the feasibility of using FMOs in a whole-cell set up but also opened the way for future applicability of these human biocatalysts in oxidation of drug candidates during the drug development process.

Concluding Remarks

Finally, in the last decade a huge effort has been made by different research groups as well as chemical companies to translate the many catalytic reactions of BVMO enzymes into large scale preparative conversions. To this end, CHMO and closely related BVMOs were used for the production of enantiopure products, mainly for application in the fragrance industry (Fink et al., 2013). In 2006 a company filed a patent for a process for the preparation of modafinil, a wakefulness-promoting drug used in the treatment of shift work sleep disorders (Riva et al., 2006) using PAMO. This enzyme is able to convert the sulfide precursor to the correct enantiomer of this sulfoxide. More recently, another company has filed a patent for the production of the proton pump inhibitor esomeprazole by CHMO (Bong et al., 2013). The company used this enzyme to improve methods to manufacture chiral sulfoxides, which are important molecules for pharmaceutical synthesis. Their biocatalytic processes using BVMOs, improves enantiometric purity and reduces sulfone impurities.

Given the importance of optically active lactones and sulfoxides in chemistry and medicine, together with the drive for less hazardous and more environmentally friendly reactions, the future looks very promising for flavoprotein monooxygenases. Although huge progress has been made for the use of these enzymes in large scale chemical syntheses, further improvement in the creation of stable and more selective monooxygenases is required in order for biocatalysis to win over pure chemical synthesis.

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Table 1: Examples of stereoselective reactions catalyzed by BVMO enzymes.

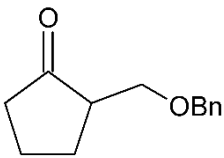
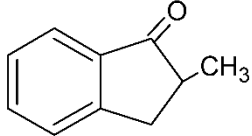
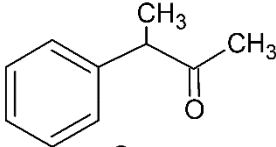
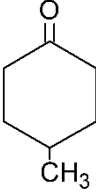
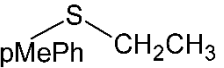
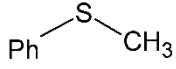
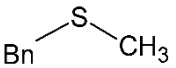
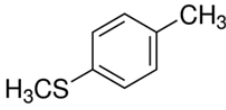
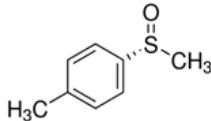
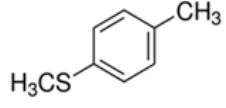
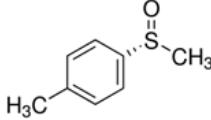
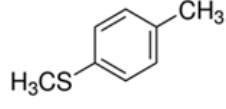
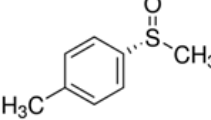
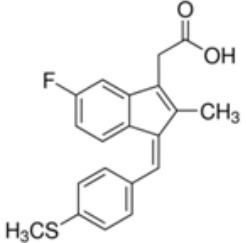
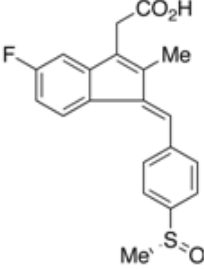
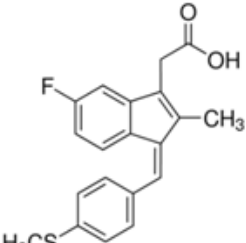
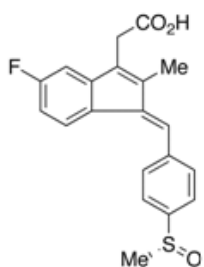
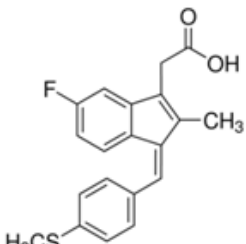
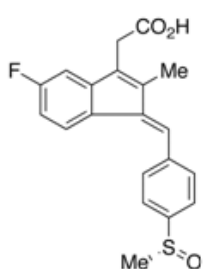
| Enzyme | Substrate | Yield(%) | %R | %S | REFERENCE |
|--------|---|----------|----|----|-------------------------|
| CHMO |  | 85 | 96 | 4 | Berenzina et al., 2002 |
| PAMO |  | 84 | 82 | 18 | Gutierrez et al., 2005 |
| HAPMO |  | 84 | 14 | 86 | Rodriguez et al., 2010 |
| CPMO |  | 68 | 46 | 54 | Iwaki et al., 2002 |
| CHMO | tertBu—S—S—tertBu | 90 | 97 | 3 | Colonna et al., 2001 |
| CHMO |  | n.r. | 27 | 73 | Light et al., 1982 |
| PAMO |  | 94 | 44 | 56 | De Gonzalo et al., 2006 |
| HAPMO |  | 55 | 15 | 85 | De Gonzalo et al., 2006 |

Table 2: Stereoselective sulfoxidations catalyzed by mammalian FMO enzymes.

| Enzyme | Substrate | product | ee |
|---------------------------------|---|--|-------|
| cDNA-expressed rabbit FMO1 |  |  | > 99% |
| cDNA-expressed rabbit FMO2 |  |  | > 99% |
| Rabbit liver microsomes |  |  | 96% |
| Mini-pig liver FMO1 |  |  | 98% |
| Rabbit lung FMO2 |  |  | 99% |
| cDNA-expressed human liver FMO3 |  |  | 87% |

(a) Rettie et al., 1994

(b) Hamman et al., 2000

Figure 1 Access channel comparisons for flavoprotein monooxygenases. A, bacterial FMO from *Methylophaga*, volume 2981 Å³ (Protein Data Bank Code 2VQ7, Alfieri et al., 2008); B, yeast FMO from *S. pombe*, volume 2330 Å³ (Protein Data Bank Code 2GV8, Eswaramoorthy et al., 2006); C, bacterial PAMO from *Thermobifida fusca*, 2367 Å³ (Protein Data Bank Code 1W4X, Malito et al., 2004); D bacterial CHMO from *Rhodococcus*, volume 1811 Å³ (Protein Data Bank Code 3GWD, Mirza et al., 2009). Volumes were calculated using the CASTp program (Dundas et al., 2006).

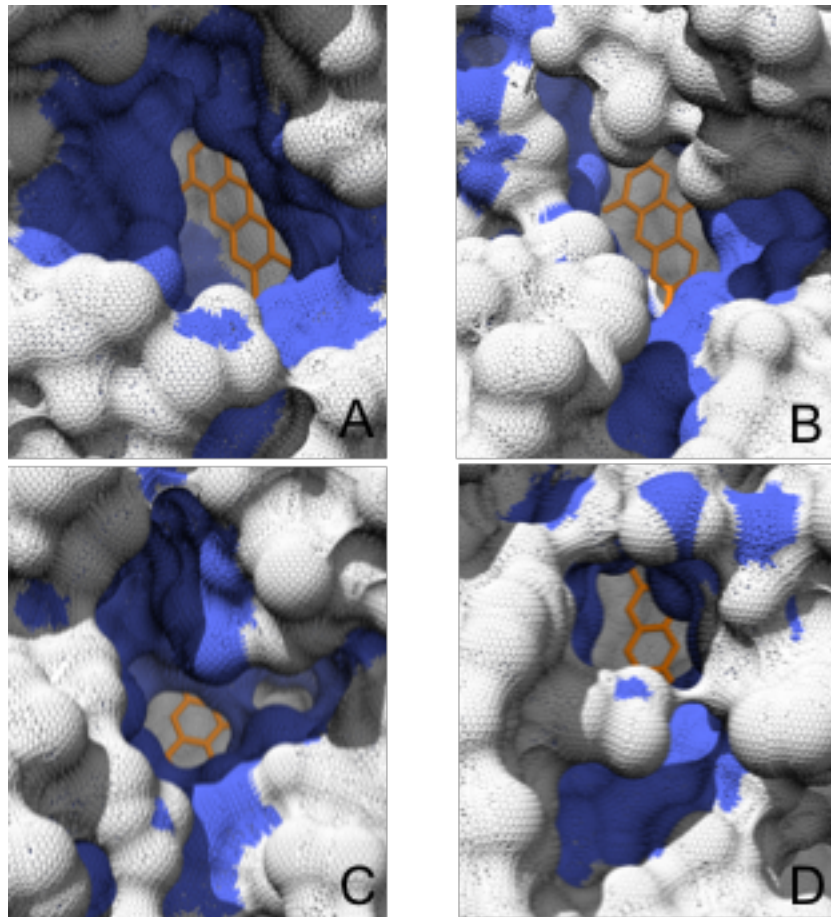


Figure 2 Scheme of the catalytic cycle of BVMO

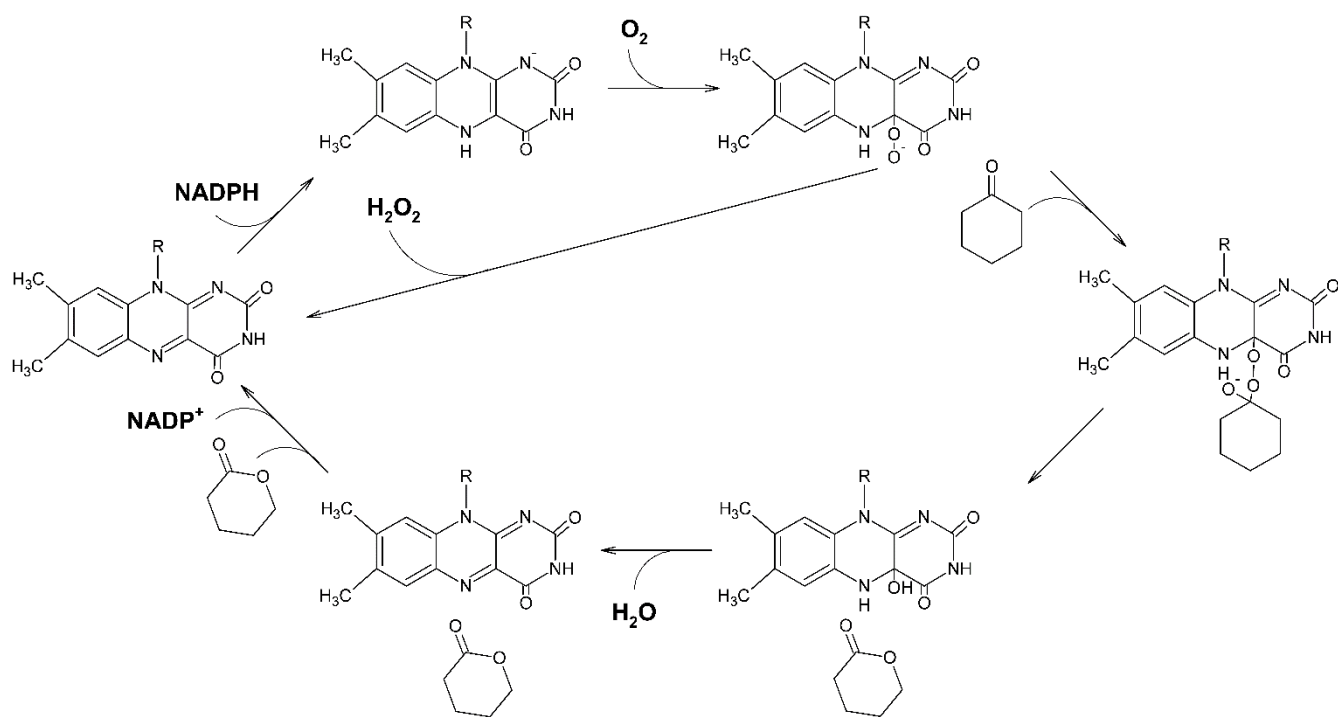


Figure 3 Selected substrates of FMO. Arrows indicate the positions of *N*- or *S*-oxygenation

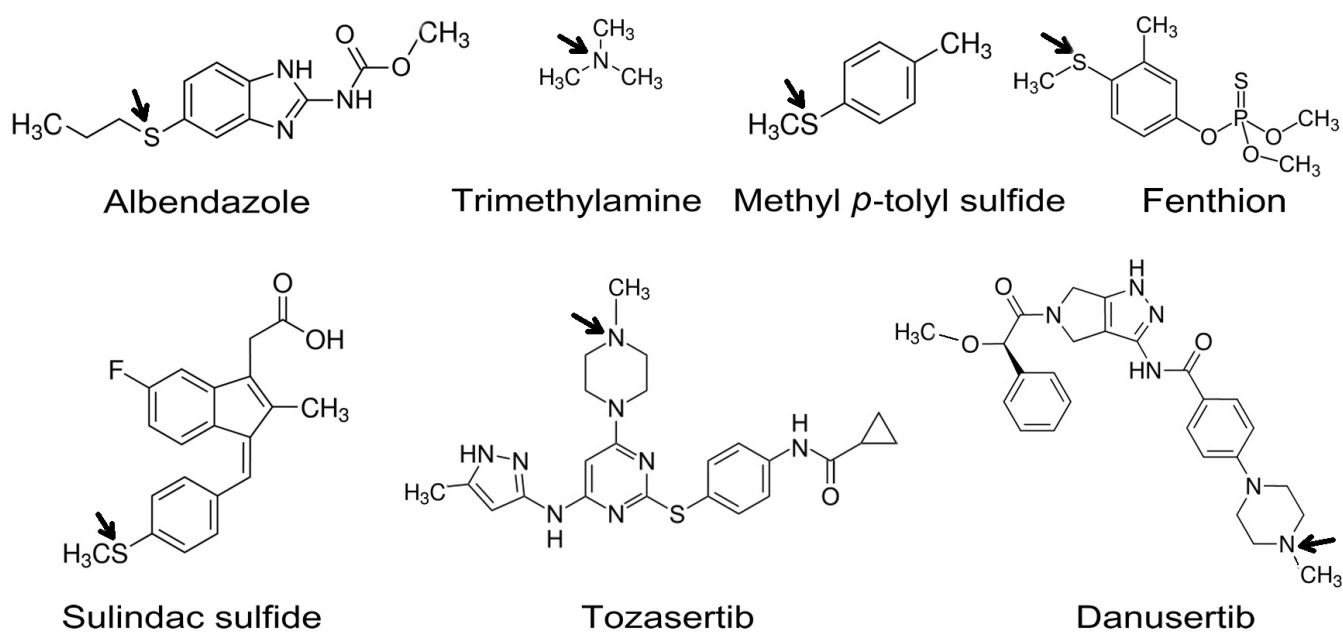


Figure 4 Biotransformation of sulindac. Racemic prodrug sulindac sulfoxide is reversibly reduced to the active sulfide metabolite, which is reoxidized by FMO to the (*R*)-sulfoxide enantiomer in high yields.

