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Review

Flavin dependent monooxygenases

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ABSTRACT

Flavin-dependent monooxygenases catalyze a wide variety of chemo-, regio- and enantioselective oxygenation reactions. As such, they are involved in key biological processes ranging from catabolism, detoxification and biosynthesis, to light emission and axon guidance. Based on fold and function, flavin-dependent monooxygenases can be distributed into eight groups. Groups A and B comprise enzymes that rely on NAD(P)H as external electron donor. Groups C–F are two-protein systems, composed of a monooxygenase and a flavin reductase. Groups G and H comprise internal monooxygenases that reduce the flavin cofactor through substrate oxidation. Recently, many new flavin-dependent monooxygenases have been discovered. In addition to posing basic enzymological questions, these proteins attract attention of pharmaceutical and fine-chemical industries, given their importance as regio- and enantioselective biocatalysts. In this review we present an update of the classification of flavin-dependent monooxygenases and summarize the latest advances in our understanding of their catalytic and structural properties.

Introduction

Flavin-dependent monooxygenases are involved in a wide range of biological processes. Many of these enzymes play a key role in the catabolism of natural and anthropogenic compounds, while others assist in the biosynthesis of hormones, vitamins and antibiotics, or help in defense strategies. Flavin-dependent monooxygenases catalyze the incorporation of one atom of molecular oxygen into the substrate, while the other oxygen atom is reduced to water. Oxygen activation in these redox enzymes typically involves the formation of a (transiently) stable flavin C4a-oxygen adduct [1]. Depending on protonation state, this peroxy species reacts with nucleophilic or electrophilic substrates thereby splitting the oxygen–oxygen bond (Fig. 1). Flavin-dependent monooxygenases catalyze among others hydroxylation, Baeyer–Villiger oxidation, sulfoxidation, epoxidation, and halogenation reactions [2]. Their high selectivity renders them attractive as regio- and enantioselective biocatalysts for the synthesis of high-value chemicals.

According to the recommendations of the nomenclature committee of the International Union of Biochemistry and Molecular Biology (http://www.chem.qmul.ac.uk/iubmb/enzyme/), flavin-dependent monooxygenases belong to oxidoreductase subclasses 1.13 and 1.14. Since the purification of lactate 2-monooxygenase (EC 1.13.12.4) in 1957 [3] and salicylate 1-monooxygenase (EC 1.14.13.1) in 1965 [4], at least 130 flavin-dependent monooxygenases have been described. Therefore, they constitute the largest family of flavoenzymes [5]. Taking their structural and functional properties into account, we proposed a division into six groups [2]. Group A–B monooxygenases (EC 1.14.13) comprise single-component enzymes that rely on NAD(P)H as external electron donor (Table 1). Group C–F monooxygenases (EC 1.14.14) need a reductase partner protein for the delivery of reduced flavin (Table 1). Being scarce and poorly characterized, internal flavoprotein monooxygenases (EC 1.13.12) were not included in the original classification [2]. Internal flavoprotein monooxygenases reduce the flavin cofactor through substrate oxidation. Growing information regarding their structure and function does now allow for putting these members in newly defined groups G and H (Table 1).

In this review we present an update of the classification of flavoprotein monooxygenases, provide a complete list of these enzymes (Table S1), and summarize the properties of selected family members. Special attention is given to the many new members of group A monooxygenases. Detailed information about the biocatalytic, mechanistic, and structural properties of already well-characterized flavin-dependent monooxygenases can be found in recent reviews [6–18].

Classification of flavin-dependent monooxygenases

The classification of flavin-dependent monooxygenases is based on structural features, protein sequence motifs, electron donor and type of oxygenation reaction [2]. An overview of the classification

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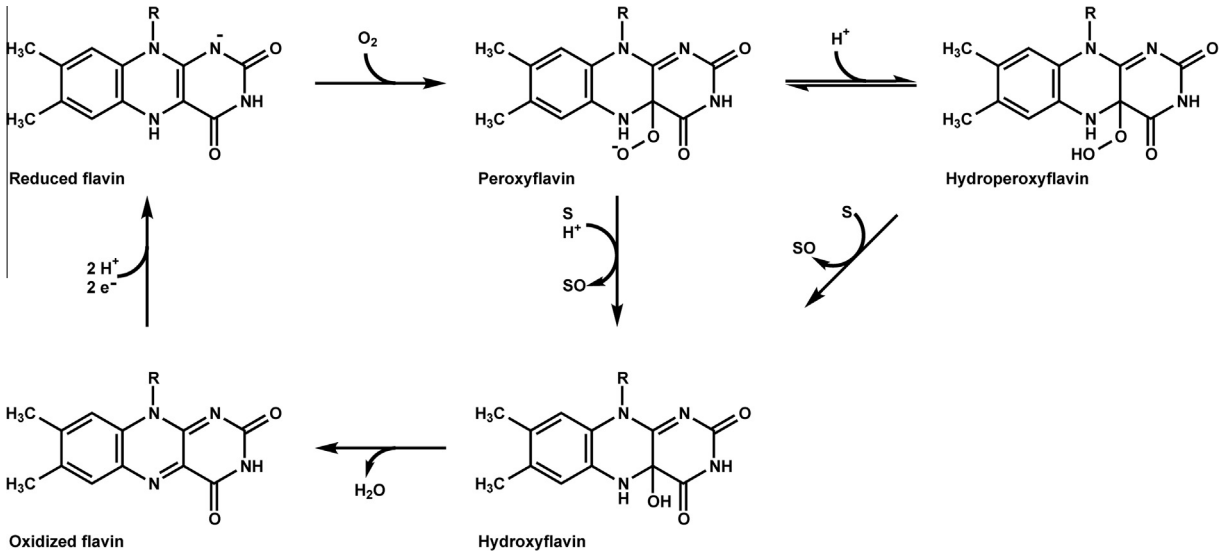


Fig. 1. General mechanism for oxygen activation in flavin-dependent monooxygenases. Reaction of reduced flavin with oxygen generates the flavin C4a-(hydro)peroxide that reacts with electrophilic or nucleophilic substrates to form the flavin C4a-hydroxide. Release of water closes the catalytic cycle.

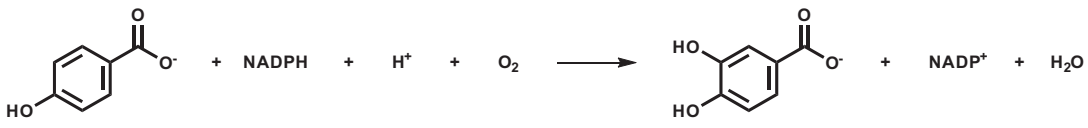
Table 1
Overview of flavin-dependent monooxygenases.

Group	Cofactor	Electron donor	Protein fold	Reaction	Prototype
A	FAD	NAD(P)H	Rossmann (GR-2)	Hydroxylation	*p*-Hydroxybenzoate 3-hydroxylase
				Sulfoxidation	MICAL
B	FAD	NAD(P)H	Rossmann (FMO)	Baeyer–Villiger oxidation	Cyclohexanone monooxygenase
				Heteroatom oxygenation	Dimethylaniline monooxygenase
				N-Hydroxylation	L-Ornithine monooxygenase
				Oxidative decarboxylation	Indole-3-pyruvate monooxygenase
C	FMN	$FMNH_2$	Tim-barrel (luciferase)	Light emission	Alkanal monooxygenase
				Baeyer–Villiger oxidation, epoxidation	Diketocamphane monooxygenase
				Desulfurization, sulfoxidation	Alkanesulfonate monooxygenase
				Hydroxylation	Long-chain alkane monooxygenase
D	FAD/FMN	$FADH_2$/$FMNH_2$	Acyl-CoA dehydrogenase	Hydroxylation	*p*-Hydroxyphenylacetate 3-hydroxylase
				N-Hydroxylation	KijD3 sugar *N*-oxygenase
E	FAD	$FADH_2$	Rossmann (GR-2)	Epoxidation	Styrene monooxygenase
F	FAD	$FADH_2$	Rossmann (GR-2)	Halogenation	Tryptophan 7-halogenase
G	FAD	Substrate	Rossmann (MAO)	Oxidative decarboxylation	Tryptophan 2-monooxygenase
H	FMN	Substrate	Tim-barrel (glycolate oxidase)	Oxidative decarboxylation	Lactate 2-monooxygenase
				Oxidative denitration	Nitronate monooxygenase

GR, glutathione reductase; FMO, flavin-containing monooxygenase; MAO, monoamine oxidase; MICAL, molecule interacting with CasL.

is given in Table 1 and the protein folds of representative prototype enzymes are presented in Fig. 2.

Group A



Group A flavin monooxygenases are encoded by a single gene, contain a glutathione reductase (GR-2) type Rossmann fold for FAD binding and use NAD(P)H as electron donor. These enzymes can be traced in the Protein database through the so-called DG fingerprint, a specific amino acid sequence motif with a dual function in both FAD and NAD(P)H binding [12,19].

Group A flavin monooxygenases are widely involved in the microbial degradation of (poly)aromatic compounds and the biosynthesis of natural products [20]. *para*-Hydroxybenzoate hydroxylase (PHBH[1]; EC 1.14.13.2) is the prototype monooxygenase of group A [21]. The enzyme catalyzes the *ortho*-hydroxylation of 4-hydroxybenzoate to give 3,4-dihydroxybenzoate:

[1] *Abbreviations used*: PHBH, *para*-hydroxybenzoate hydroxylase; BVMOs, Baeyer–Villiger monooxygenases; FMOs, flavoprotein monooxygenases; NHMOs, *N*-hydroxylating monooxygenases; OMO, ornithine monooxygenase; HPAH, 4-hydroxyphenylacetate 3-hydroxylase; HPA, 4-hydroxyphenylacetate; SMO, styrene monooxygenases; TMO, tryptophan 2-monooxygenase; LMO, lactate 2-monooxygenase; NMO, nitronate monooxygenase; P3N, propionate 3-nitronate; KMO, kynurenine 3-monooxygenase; 3HB6H, 3-hydroxybenzoate 6-hydroxylase; OnpA, *ortho*-nitrophenol 2-monooxygenase; NmoA, nonylphenol 4-monooxygenase; OpdA, octylphenol 4-monooxygenase; HspB, 6-hydroxy-3-succinoyl-pyridine hydroxylase; TmuM, trimethyluric acid monooxygenase; hMICAL1, human MICAL1 monooxygenase domain; mMICAL2, mouse MICAL2 monooxygenase domain; PAMO, phenylacetone monooxygenase; PAO, phenylalanine 2-monooxygenase; HpxO, uric acid monooxygenase.