# 7.03 Flavin-Dependent Enzymes

**Rebecca L. Fagan and Bruce A. Palfey**, University of Michigan Medical School, Ann Arbor, MI, USA

## 7.03.1 Introduction

Hundreds, perhaps thousands, of flavoenzymes have been identified and characterized.[1] Current estimates are that ~1–3% of bacterial and eukaryotic genomes encode proteins that bind flavin.[2] Flavins catalyze a wide variety of chemical transformations, and thus are involved in almost all aspects of biology: energy production, natural product synthesis, biodegradation, chromatin remodeling, DNA repair, apoptosis, protein folding, xenobiotic detoxification, and neural development. Flavoenzymes play a key role in metabolism, shuffling electrons between two-electron reactants (e.g., NADPH, fumarate, lactate) and one-electron reactants such as the metal centers and quinones of the respiratory chain. Many flavoenzymes are involved in natural product biosynthesis, such as the recently discovered RebF and RebH, which catalyze halogenation reactions leading to the antibiotic rebeccamycin.[3] A recently discovered flavoenzyme, lysine-specific demethylase 1 (LSD1), demethylates histone lysines.[4] MICAL (*m*olecule *i*nteracting with *CasL*), a multidomain protein, transmits signals that guide axonal growth. The essential N-terminal domain of this protein is a flavin-dependent monooxygenase.[5]

Flavins are derivatives of riboflavin (vitamin $B_2$) and consist of a tricyclic isoalloxazine moiety derivatized with a ribityl chain at N10 (**Figure 1**). The two common enzymatically active flavins are flavin mononucleotide (FMN), which is riboflavin phosphorylated at the 5′-OH of the ribityl chain, and flavin adenine dinucleotide (FAD), which is the condensation product of FMN and AMP. The ribityl chain and its 5′-OH modification is generally a handle for binding by proteins, but occasionally an –OH group participates in catalysis.[6] In most cases, flavoenzymes are nondissociable noncovalent complexes of apoprotein and flavin, with dissociation constants in the nanomolar range or lower. Therefore, under most circumstances, the flavin can be thought of as part of the enzyme. In contrast to the usual noncovalent complexes, there are many cases where the flavin is covalently attached to the enzyme. Covalent attachment occurs at either the 8-$\alpha$ (methyl) or the 6-position of the isoalloxazine, and sometimes at both sites (see **Figure 1**), through either of the imidazole nitrogens of histidine, the sulfur of cysteine, or the phenolic oxygen of tyrosine. The need for covalent attachment is not fully understood, since there are examples of enzymes (e.g., cholesterol oxidases from different species) that use covalently and noncovalently bound flavins to accomplish the same chemistry.[7] In some cases, the redox potential of the flavin is increased markedly (~100 mV) by covalent attachment.[8] Covalent attachment could protect the flavin from deleterious modifications. For instance, covalent attachment of trimethylamine dehydrogenase at C6 of the FAD prevents modification to the 6-hydroxy derivative, which has been proposed to

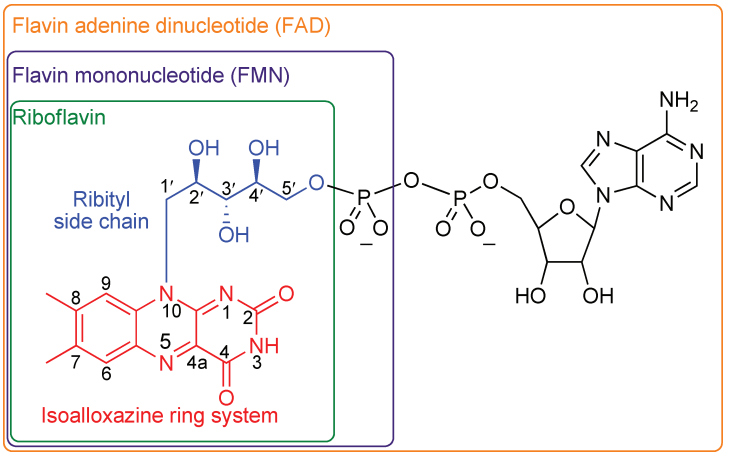



**Figure 1** Flavin structure and nomenclature.

occur by the reaction with either hydroxide or $O_2$.[9,10] The other extreme of binding is also known – sometimes, flavins bind so weakly to enzymes that they act as substrates rather than prosthetic groups. For example, bacterial luciferase uses FMN as a substrate after it is reduced by pyridine nucleotide in a reaction catalyzed by a separate enzyme.

The chemically active portion of flavins is the heterocyclic isoalloxazine ring system, which can have three stable oxidation states: fully oxidized, semiquinone (1-electron reduced), and hydroquinone (2-electron reduced) (**Figure 2**). The oxidized isoalloxazine ring system is planar in solution and in most protein structures, but is distorted in a few protein structures, doubtlessly influencing its ability to accept one or two electrons. This electron-deficient heterocycle is susceptible to nucleophilic attack at N5, C4a, and C6. Attack at C6 is not involved in catalysis – indeed, it can be an enzyme-inactivating side reaction – but is important in the covalent flavinylation during the posttranslational maturation of a few flavoenzymes. Oxidized isoalloxazines are highly polarizable, so the electron distribution – and, therefore, reactivity – is influenced greatly by interactions with proteins. The p$K_a$ of N3, which is ~10 in aqueous solution, may become quite low in an enzyme, but is not often suggested to be a major determinant of flavin reactivity.

The single-electron reduced state, the semiquinone, is thermodynamically unstable in aqueous solution, with only ~5% present in equimolar mixtures of fully oxidized and fully reduced flavins.[11] However, protein interactions alter the stability of the semiquinone markedly – some stabilizing it, others suppressing it.[12] The

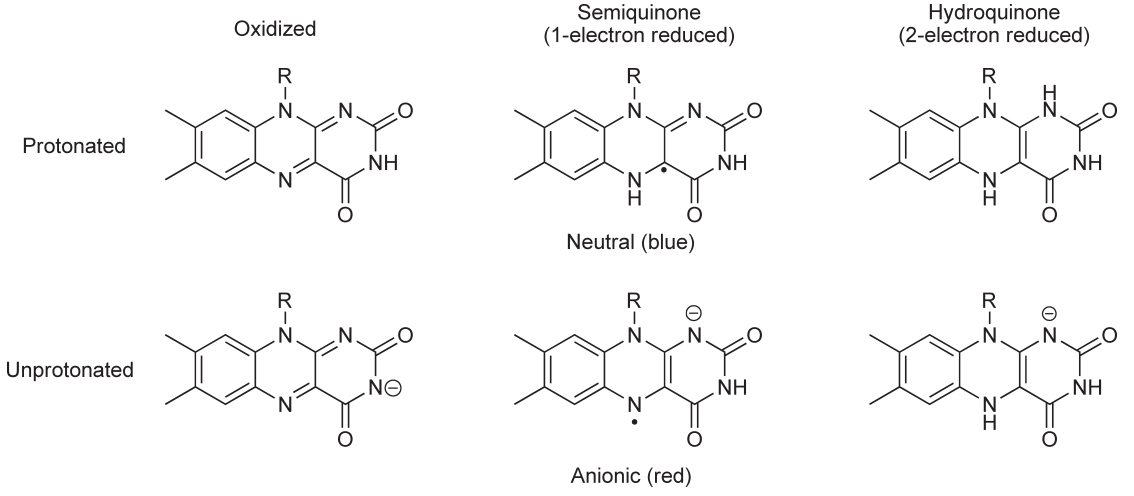


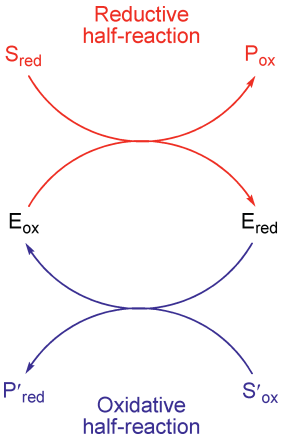

**Figure 2** Chemical states of flavin.

unpaired electron is delocalized over the highly conjugated isoalloxazine.[13,14] High spin density is observed by electron paramagnetic resonance (EPR) spectroscopy at C4a for the neutral (blue) radical. When deprotonated, high spin density is on N5 of the red anion. The p$K_a$ of N5 is ~8.5 in aqueous solution and can vary markedly in an active site, so either protonation state can be important in enzyme reactions. Semiquinones react by single-electron oxidations or reductions, and can also couple to other radicals.

The two-electron reduced, or hydroquinone, isoalloxazine is pale yellow. It is an electron-rich heterocycle and, when planar, is antiaromatic according to Hückel's rule. The ring system of the hydroquinone in some small-molecule structures and some protein structures is bent by as much as 30° along the N5–N10 axis,[15] presumably to relieve the antiaromaticity. However, the majority of hydroquinones bound to proteins do not deviate from planarity much more than oxidized isoalloxazines. This is likely to be influenced by protonation of N1, whose p$K_a$ is ~6.7 in aqueous solution. Quantum calculations show that neutral hydroquinone adopts butterfly conformation but the anion is planar.[16–18] A survey of crystal structures agrees with this correlation – anionic hydroquinones are generally nearly planar, while the few instances of the butterfly conformation belonged to neutral hydroquinones.[19] Hydroquinones react as single-electron donors, as hydride donors, or as nucleophiles at N5 or C4a.

Redox potentials indicate the ability of the flavin to accept electrons. Therefore, this important property provides information on reactivity toward redox substrates and also is a gauge of the electrophilicity or nucleophilicity of the isoalloxazine. The redox potential, like other properties, can be tuned by the protein. The two-electron redox potential of free flavin at pH 7 is −207 mV, while the single-electron potentials are −314 mV for the oxidized/semiquinone couple and −124 mV for the semiquinone/hydroquinone couple.[20] All potentials in this chapter are relative to the standard hydrogen electrode. One- and two-electron redox potentials of flavoprotein span a remarkable range: from −495 mV for the semiquinone/reduced couple in *Azotobacter vinelandii* flavodoxin[21] to +153 mV for the oxidized/semiquinone couple of electron-transfer flavoprotein from *Methylophilus methyltrophus*.[22] The highly conjugated isoalloxazine heterocycle is very polarizable, causing its electron distribution to be altered markedly by nearby charges, hydrogen bonds, pi-stacking with aromatics, or other van der Waals interactions. Interactions that lower electron density in the conjugated diimine of the oxidized isoalloxazine will increase the redox potential. Interactions between the hydroquinone and the protein influence the electron distribution of the electron-rich enediamine, modulating the reactivity. Stabilizing the negative charge of the anionic reduced flavin increases the redox potential.

The catalytic cycles of flavoenzymes can usually be divided into reductive and oxidative half-reactions (**Scheme 1**). In the reductive half-reaction, the oxidized flavoprotein is reduced by the first substrate, resulting in reduced flavoenzyme. In the oxidative half-reaction, a second substrate oxidizes the reduced flavoprotein (**Scheme 1**), usually after the product of the reductive half-reaction dissociates, so flavoenzymes frequently have ping-pong kinetic mechanisms. Even when the first product remains bound during the oxidative




**Scheme 1** Typical catalytic cycle of flavoproteins.