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Flavoproteins - Natural Supramolecules for Biocatalysis

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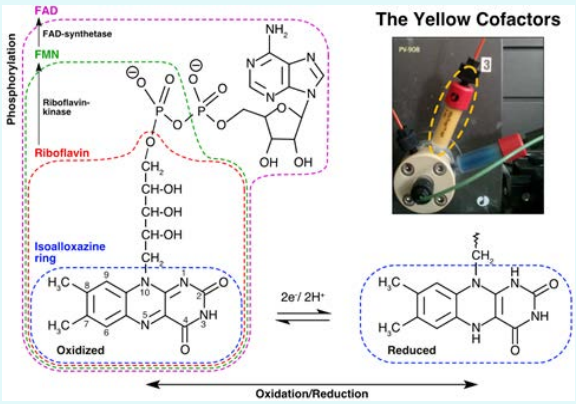
COLUMN ARTICLE

Our research focuses on the identification and characterization of novel flavoproteins in order to turn them into valuable biocatalysts. Flavin-dependent enzymes (flavoproteins, flavoenzymes) are pervasive among animals, plants and microorganisms catalyzing redox reactions. Containing a flavin nucleotide (flavin adenine dinucleotide FAD or flavin mononucleotide FMN) as a prosthetic group or cofactor, flavoenzymes are used for electron transfer reactions where the flavin acts as the electron mediator. In most cases flavins are non-covalently bound to the apoprotein. Considering the flavin as a guest within the active site of the host molecule (protein) one can speak of a supra-molecular compound. Thereby, the flavin is bound to the protein through a plurality of interactions like hydrogen bondings, hydrophobic effects (with water molecules) or Van der Waals forces.

The small vitamin B2 molecule (riboflavin) is synthesized by the organism as a building block for the production of FMN and FAD (scheme). The phosphorylation of the ribityl side chain is performed by a riboflavin kinase, while the subsequent adenylation is catalyzed by an FAD-synthetase [1-3]. The reactive moiety of the flavin is a tricyclic isoalloxazine ring, which becomes oxidized or reduced at the N5-position. Due to this ring system flavoproteins have the possibility to perform one or two electron transfer reactions and also dioxygen activation [4]. These properties turn the flavin into a valuable part of many biocatalytic relevant enzymes and thus understanding this cofactor is important for biotechnology!



Scheme: Structure of the yellow cofactors. The isoalloxazine ring (blue) is shown in its oxidized (numbering of ring atoms indicated) and reduced state after a two-electron reduction. Synthesis of FAD (purple) and FMN (green) from riboflavin (red) is performed by an FAD-synthetase or riboflavin kinase, respectively. The inlet picture shows a yellow dyed column during purification of a flavin-containing protein.

Flavins research began in 1932 through the isolation of the so-called "Gelbe Ferment" from brewer`s bottom yeast (*Saccharomyces pastorianus*) [5]. During investigations of oxidation reaction mechanisms an enzyme/coenzyme system was found, which was involved in the transfer of hydro-

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gen. The liquid substance could be separated in a colorless and another yellow fraction. Two years later, the Swede H. Theorell further investigated the "Gelbe Ferment" as a guest researcher at the Kaiser Wilhelm Institute Berlin together with O. Warburg.

Theorell discovered that the "Gelbe Ferment" consists of a specific protein and a phosphorylated riboflavin [6]. He received the Nobel Prize in Physiology/Medicine in 1955 for the discovery of the oxidation enzyme.

Since, the number of "yellow" enzymes (= flavin-containing) continuously increased. The progress in molecular cloning, complete genome sequencing, bioinformatics and determination of crystal structures led to detailed characterizations of many "yellow" enzymes and hitherto to structural classification of flavoprotein subclasses [7-10]. The large reaction diversity of flavin-dependent enzymes contains dehydrogenation, oxidation, monooxygenation, halogenation, and reduction (disulfides and double bonds) [2,11].

Currently we investigate the following flavoenzymes: (I) the reduction of carbon double bonds by old yellow enzymes (OYEs, EC 1.6.99.1), (II) monooxygenation (epoxidation, sulfoxidation) by styrene monooxygenases (SMOs, EC 1.14.14.11 and EC 1.5.1.36) for chiral synthesis, (III) N-hydroxylation by BVMO-like monooxygenases as part of siderophore biosynthesis (e.g. EC 1.14.13.195 and related), (IV) aromatic ring hydroxylases as PHBH (EC 1.14.13.2) and related enzymes within aromatic degradation pathways, and (V) azo dye degradation by FMN containing azoreductases (EC 1.7.1.6).

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CONFLICT OF INTEREST

The authors declare that no financial interest or any conflict of interest exists.

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