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Review article

The family of berberine bridge enzyme-like enzymes: A treasure-trove of oxidative reactions

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ABSTRACT

Biological oxidations form the basis of life on earth by utilizing organic compounds as electron donors to drive the generation of metabolic energy carriers, such as ATP. Oxidative reactions are also important for the biosynthesis of complex compounds, *i.e.* natural products such as alkaloids that provide vital benefits for organisms in all kingdoms of life. The vitamin B_2-derived cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) enable an astonishingly diverse array of oxidative reactions that is based on the versatility of the redox-active isoalloxazine ring. The family of FAD-linked oxidases can be divided into subgroups depending on specific sequence features in an otherwise very similar structural context. The sub-family of berberine bridge enzyme (BBE)-like enzymes has recently attracted a lot of attention due to the challenging chemistry catalyzed by its members and the unique and unusual bi-covalent attachment of the FAD cofactor. This family is the focus of the present review highlighting recent advancements into the structural and functional aspects of members from bacteria, fungi and plants. In view of the unprecedented reaction catalyzed by the family's namesake, BBE from the California poppy, recent studies have provided further insights into nature's treasure chest of oxidative reactions.

Contents

Abbreviations: 6HDNO, 6-hydroxy-D-nicotine oxidase; A40926, teicoplanin homolog; CpADHAPS, alkyldihydroxyacetonephosphate synthase from *Cavia porcellus*; DdADHAPS, alkyldihydroxyacetonephosphate synthase from *Dictyostelium discoideum*; AknOx, aclacinomycin-N/aclacinomycin-A oxidase; AldO, alditol oxidase; *At*, *Arabidopsis thaliana*; *At*CKX, cytokinin dehydrogenase from *Arabidopsis thaliana*; BBE, berberine bridge enzyme; BG60, pollen allergen; CBDAS, cannabidiolic acid synthase; ChitO, chitooligosaccharide oxidase; CholOx, cholesterol oxidase; cnsA, aurantioclavine synthase; *Cv*, *Chlorella variabilis*; DLDH, D-lactate dehydrogenase; DprE, decaprenylphosphoryl-beta-D-ribose oxidase; E22O, Ecdysteroid-22-oxidase; EasE, chanoclavine synthase; *Ec*, *Eschscholzia californica*; EncM, FAD-dependent oxygenase; EugO, eugenol oxidase; FOX, flavin-dependent oxidoreductase; FsqB, fructosyl amino acid oxidase; GilR, pregilvocarcin V oxidase; GLDH, L-galactono-1,4-lactone dehydrogenase; GOOX, glucooligosaccharide oxidase; *Ha*-CHOX, *Helianthus annuus* carbohydrate oxidase; HMM, hidden markov model; HOX, hexose oxidase; HPM9, alcohol oxidase from *Hypomyces subiculosus*; ICN, indole carbonyl nitrile; LaO, carbohydrate oxidase; *Ls*-CHOX, *Lactuca sativa* carbohydrate oxidase; *Mp*, *Marchantia polymorpha*; *Mt*, *Myceliophthora thermophile*; MurB, UDP-N-acetylenolpyruvoylglucosamine reductase; *N. rileyi*, *Nomuraea rileyi*; Nec5, nectarin 5; *Nf*, *Neosartorya fumigate*; *Nt*, *Nicotiana tabacum*; PCMH, *p*-cresol methylhydroxylase; PFAM, protein family; Phl p 4, major pollen allergen (glucose dehydrogenase); *Pi*, *Phytotropha infestans*; *Pp*, *Physcomitrella patens*; RPA1076, oxidoreductase from *Rhodopseudomonas palustris*; SCOPe, structural classification of proteins – extended; *Sm*, *Selaginella moellendorffii*; STOX, (*S*)-tetrahydroprotoberberine oxidase; TamL, tirandymycin oxidase; THCAS, tetrahydrocannabinolic acid synthase; VAO, vanillyl-alcohol oxidase; *Vc*, *Volvox carteri f. nagariensis*; XEG, xylanendoglucanase; XylO, xylooligosaccharide oxidase; *Zm*CKO2, cytokinin oxidase from *Zea mays*; *Zm*CKX1, cytokinin oxidase from *Zea mays*.

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1. Introduction

BBE-like enzymes form a subgroup of the superfamily of FAD-linked oxidases (SCOPe d.58.32) that is structurally characterized by a typical fold observed initially for vanillyl-alcohol oxidase (VAO) [1]. Therefore, the structural architecture has been described initially as the VAO-fold [2] and all members of this superfamily share a common architecture that can be divided into a FAD-binding domain and a substrate binding domain, which is also referred to as the cap domain [1]. A comprehensive review on the first identified members of this family was published by Leferink et al. [3]. In recent years, however, the rapidly growing number of member proteins has revealed a relatively low sequence conservation and an astonishing diversity of catalyzed chemical reactions. To account for these observations the superfamily of FAD-linked oxidases can be divided into subfamilies that better reflect structural, functional and evolutionary aspects of individual group members. The family of BBE-like enzymes forms a relatively large subgroup featuring a characteristic C-terminal structural element following the substrate binding region (see below). Considering the differences in chemical reactivities observed for the relatively few enzymes that are characterized in detail, additional subclassifications are helpful and will be introduced in this review.

The namesake of this family is the (*S*)-reticuline oxidase or berberine bridge enzyme from California poppy (*Eschscholzia californica*) that catalyzes the conversion of (*S*)-reticuline to (*S*)-scoulerine by mediating an oxidative ring closure reaction. The C-C bond that is formed in this reaction is referred to as the berberine bridge and marks a branch point in the biosynthesis of benzylisoquinoline alkaloids [4] (Fig. 1).

Together with glucooligosaccharide oxidase (GOOX) from *Acremonium strictum* [5,6], the characterization of BBE [7–9] revealed one interesting hallmark of the family of BBE-like proteins: a bicovalently attached FAD cofactor. The characteristic 6-*S*-cysteinyl-, 8α-*N*1-histidyl-FAD linkage is only found in the BBE-subfamily of the FAD-linked oxidases and has been shown to increase the redox potential of the cofactor by more than 300 mV compared to free flavin [10]. How exactly this is linked to the individual reactions catalyzed is still not very well understood, especially since some family members appear to have lost the cysteinyl linkage. Additional highlights of BBE-like family members are the recently identified flavin N5-oxides observed in EncM [11] and challenging carbon-carbon bond formation reactions involving complex biomolecules in natural product biosynthesis [12,13]. In comparison to the rapidly growing number of group members identified in various sequencing projects, the functional characterization of individual proteins is severely lagging behind also due to the challenge of identifying the true *in vivo* substrates, as exemplified in Ref. [14]. Considering the stunning reactions catalyzed by members the BBE-like enzyme family that have been analyzed in detail so far, it will be interesting to witness which new surprising functional details of yet uncharacterized family members will be discovered in the future.

2. Structural characteristics of BBE-like proteins

2.1. Differentiation from other families of FAD-linked oxidases

As mentioned in the introduction, BBE-like enzymes are a subfamily of the large superfamily of FAD-linked oxidases (SCOPe d.58.32). Characteristic structural features of this family are a FAD binding module that is formed by the *N*- and *C*-terminal parts of the protein, and a substrate binding module that, together with the isoalloxazine ring of FAD, provides the environment for efficient substrate binding and oxidation (Fig. 2). While the overall fold of BBE-like proteins is similar to that of other prototypic members of this superfamily, a specific structural feature in the vicinity of the FAD-binding site distinguishes the BBE family from other FAD-linked oxidases such as vanillyl alcohol oxidase (VAO)-like enzymes (SCOPe d.58.32.1), the D-lactate dehydrogenase family (SCOPe d.58.32.2), cholesterol oxidase-like enzymes (SCOPe d.58.32.3), cytokinin dehydrogenases (SCOPe d.58.32.4) and alditol oxidase-like enzymes (SCOPe d.58.32.6).

The *N*-terminal part of the hidden Markov model (HMM) specific for the BBE and BBE-like enzymes (pfam entry: PF08031, [15]) highlights a characteristic Y/FxN motif (Fig. 3A). This element contains a highly conserved aromatic residue (Tyr or Phe), which in case of a Tyr interacts with the N1-C2=O locus of the isoalloxazine ring and a strictly conserved Asn residue that hydrogen bonds with the before mentioned Tyr side chain. This arrangement sterically influences the positioning of the ribityl side chain of the cofactor as well as a structural element (residues 169–179 – *Ec*BBE numbering) involved in forming part of the oxygen binding pocket on the *re*-side of the isoalloxazine ring (Fig. 3C) [16]. Additionally, Asn458 is important for the positioning of His174 that stabilizes the negative charge N1-C2=O locus of the reduced flavin and has been shown to be involved in the formation of the C6-cysteinyl linkage [20].

The remaining conserved features of the HMM logo of the BBE-like protein pfam entry correspond to structurally relevant residues of the *C*-terminal FAD-binding region and are therefore also present in other groups of the superfamily. However, an additional important structural element that is directly coupled to the Y/FxN motif features another highly conserved element of the BBE-like protein family. In fact, the region closing the active site cavity at its rear part features a conserved salt bridge between Asp192 and Arg409. The