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Diversity of structures, catalytic mechanisms and processes of cofactor biosynthesis of tryptophylquinone-bearing enzymes

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ABSTRACT

Tryptophyquinone-bearing enzymes contain protein-derived cofactors formed by posttranslational modifications of Trp residues. Tryptophan tryptophylquinone (TTQ) is comprised of a di-oxygenated Trp residue, which is cross-linked to another Trp residue. Cysteine tryptophylquinone (CTQ) is comprised of a di-oxygenated Trp residue, which is cross-linked to a Cys residue. Despite the similarity of these cofactors, it has become evident in recent years that the overall structures of the enzymes that possess these cofactors vary, and that the gene clusters that encode the enzymes are quite diverse. While it had been long assumed that all tryptophylquinone enzymes were dehydrogenases, recently discovered classes of these enzymes are oxidases. A common feature of enzymes that have these cofactors is that the posttranslational modifications that form the mature cofactors are catalyzed by a modifying enzyme. However, it is now clear that modifying enzymes are different for different tryptophylquinone enzymes. For methylamine dehydrogenase a di-heme enzyme, MauG, is needed to catalyze TTQ biosynthesis. However, no gene similar to *mauG* is present in the gene clusters that encode the other enzymes, and the recently characterized family of CTQ-dependent oxidases, termed LodA-like proteins, require a flavoenzyme for cofactor biosynthesis.

1. Introduction

Tryptophylquinone enzymes possess tryptophan residues that have undergone posttranslational modifications that enable them to participate in catalysis and redox reactions. They are members of a broader group of enzymes that contain a variety of protein-derived cofactors [1], in which catalytic and redox centers of the enzymes are formed from irreversible posttranslational modification of one or more amino acid residues. Two types of tryptophan-derived cofactors have been identified (Fig. 1), tryptophan tryptophylquinone (TTQ) [2] and cysteine tryptophylquinone (CTQ) [3]. In both TTQ and CTQ, two oxygen atoms are inserted into the indole ring of a specific Trp residue. In TTQ, this modified side chain is cross-linked to the indole ring of another Trp. In CTQ, it is cross-linked to a Cys sulfur. The tryptophylquinone enzymes that have been characterized thus far are similar in that each oxidizes primary amines. Methylamine dehydrogenase (MADH) [4] and aromatic amine dehydrogenase (AADH) [5] possess TTQ. Quinohemoprotein amine dehydrogenase (QHNDH) possesses CTQ, as well as two *c*-type hemes [3]. The lysine ε-oxidase, LodA [6], and the glycine oxidase, GoxA [7], each possess CTQ. There are striking differences in the overall structures of these enzymes. However, comparison of these structures reveals that features of the active sites of the enzymes are conserved. While the initial steps in the reactions catalyzed by the tryptophylquinone enzymes are similar, there are some notable differences in the overall reaction mechanisms. The genes present in the operons that encode the enzymes vary considerably. While each of these enzymes requires a modifying enzyme to catalyze posttranslational modifications that form the quinone cofactor, the nature of modifying enzyme varies depending upon the enzyme that bears the tryptophylquinone cofactor.

2. Structures of tryptophylquinone enzymes

Crystal structures for the TTQ-containing enzymes, MADH and AADH, and the CTQ-containing enzymes, QHNDH, LodA and GoxA, have been solved to high resolution (Table 1 and references therein).

Abbreviations: AADH, aromatic amine dehydrogenase; CTQ, cysteine tryptophylquinone; KIE, kinetic isotope effect; QHNDH, quinohemoprotein amine dehydrogenase; LTQ, lysine tyrosylquinone; MADH, methylamine dehydrogenase; MIO, 4-methylideneimidazole-5-one; TPQ, 2,4,5-trihydroxyphenylalanine-quinone; TTQ, tryptophan tryptophylquinone

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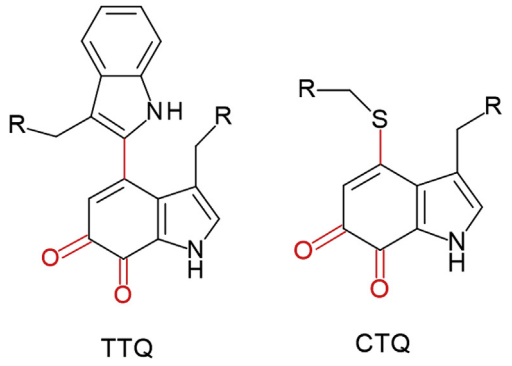

Fig. 1. Tryptophan tryptophylquinone (TTQ) and cysteine tryptophylquinone (CTQ). The posttranslational modifications of the residues are shown in red and R indicated point on the protein where the residues are attached.

AADH (Fig. 2A) and MADH (Fig. 2B) are $\alpha_2\beta_2$ heterotetramers with similar structures (rmsd = 2.2 Å for α-carbons) and high sequence identity in both α (40%) and β (23%) chains. The TTQ cofactor (Fig. 2D and E) in each of these enzymes is formed between two Trp residues in the smaller β subunit. QHNDH is an αβγ heterotrimer (Fig. 2C) with the active site formed from Cys and Trp residues on the γ subunit (Fig. 2F). The α subunits of MADH and AADH are structurally similar to the β subunit of QHNDH, each forming a 7-bladed beta-propeller, while the quinone-containing subunits share no similarity. The α subunit of QHNDH is composed of four domains. The N-terminal domain (αd1) houses two *c*-type hemes. The remaining α domains are comprised of antiparallel beta-barrels.

LodA from *Marinomonas mediterranea* and GoxA from *Pseudoalteromonas luteoviolacea* are currently the only structures of a recently identified group of CTQ-containing enzymes called LodA-like proteins, which are widespread among bacterial species [8]. They share a novel fold featuring a large α-helical region and a beta-barrel (Fig. 3A). GoxA contains an additional alpha helical domain of unknown function while LodA contains two long "arms" consisting of two antiparallel beta strands. Crystallographically, both proteins form homotetramers (Fig. 3B and C), although only GoxA exhibits a molecular weight consistent with this in size exclusion chromatography [7], while LodA is predicted to exist as a homodimer in solution [9]. Interestingly, the subunit interfaces are completely different between the proteins. In LodA, two possible dimer interactions are mediated by the "arms" mentioned above. These structures are completely absent in GoxA, which instead exhibits tight intersubunit interactions to form a dimer of dimers. A particularly intriguing feature of the GoxA structure is the insertion of a loop of one subunit into the active site of another. Tyr766 and His767 of this loop interact with the CTQ cofactor through water molecules, generating a very small, buried active site (Fig. 3D). This is in contrast to the relatively open active site of LodA (Fig. 3E). The size of the active site pocket as determined by quaternary structure is thus a likely contributor to the high specificity of LodA and GoxA for lysine and glycine, respectively.

3. Catalytic mechanisms of tryptophylquinone enzymes

The kinetic and chemical reaction mechanisms of MADH and AADH have been characterized (Fig. 4). Each exhibits a ping-pong kinetic mechanism with discrete reductive and oxidative half-reactions. The first step is the formation of a covalent imine (substrate Schiff base) adduct between the primary amino group of the substrate and TTQ. This is followed by abstraction of a proton from the α carbon of the bound substrate by an as yet unidentified active-site base. This proton abstraction results in reduction of TTQ and formation of the product Schiff base. Hydrolysis of the product Schiff base releases the aldehyde product and leaves a reduced aminoquinol form of TTQ [10]. This intermediate is then oxidized by the electron acceptor, converting the aminoquinol to an iminoquinone. The substrate-derived ammonia product is either released by hydrolysis or is displaced imine exchange with direct substrate Schiff base formation with another molecule of substrate in the steady-state reaction [11,12]. The electron acceptors are Type 1 copper proteins, amicyanin [13] for MADH and azurin for AADH [14]. MADH and AADH each exhibited an anomalously large deuterium kinetic isotope effect (KIE) of 17.2 [15] and 11.7 [16], respectively, on the rate of reduction of TTQ by substrate in single-turnover kinetics studies. This indicates that the proton abstraction in the reductive half-reaction occurred by quantum mechanical proton tunneling [17].

Analogous kinetic studies of QHNDH are complicated by the fact that QHNDH possesses not only CTQ, but also two covalent *c*-type hemes. As such, the electron acceptors for the reduced CTQ are present in the same enzyme, making it difficult to distinguish the reductive and oxidative half-reactions. Transient kinetic studies of the CTQ-dependent reduction of heme in QHNDH by amine substrates yielded deuterium KIE values of 3.9 and 8.5, respectively, for the reactions with butylamine and benzylamine [18], indicating that the abstraction of a proton from the α-methylene group of the substrate is at least partially rate-limiting the CTQ-dependent reduction of hemes in QHNDH by these amine substrates. In this respect, QHNDH is similar to MADH and AADH.

LodA and GoxA are oxidases rather than dehydrogenases. LodA has also been shown to exhibit a ping-pong kinetic mechanism with lysine and O_2 as substrates [19]. The reaction of GoxA, however, is more complicated. This enzyme does not obey Michaelis-Menten kinetics but shows cooperativity towards the glycine substrate [7,20]. In contrast to the dehydrogenases, the rate of reduction of CTQ by deuterated glycine by GoxA exhibited a negligible primary KIE of 1.08 [7]. Also in contrast to the dehydrogenases, it was shown for GoxA that the reduced CTQ-product adduct is not immediately hydrolyzed after the reductive half-reaction. It remains bound, and it is not released until it is exposed to O_2 [7]. Future studies will hopefully determine the basis for this resistance to hydrolysis and the mechanism by which O_2 facilitates hydrolysis.

4. Organization of gene clusters encoding tryptophylquinone enzymes

The diversity in the overall structures of tryptophylquinone enzymes is reflected in the gene clusters, which contain the genes encoding the structural proteins (Fig. 5).

MADH. The genes that encode the structural subunits of MADH are present in a relatively large methylamine utilization (*mau*) gene cluster [21]. The *mau* cluster of *P. denitrificans*, as well as most other bacteria which express MADH, contains 11 genes [22]. The genes *mauB* and *mauA*, encode the α and β subunits of MADH, respectively. Amicyanin, the electron acceptor for reduced MADH is encoded by *mauC* [23]. Four additional genes are required for expression of active MADH, *mauF* [24], *mauE* [25], *mauD* [25] and *mauG* [22]. It should be noted that expression of active MADH requires not only biosynthesis of TTQ, but also formation of six disulfide bounds on the 131 residue TTQ-bearing β subunit and translocation of the subunits to the periplasm. The functions of *mauF* and *mauE* are unknown. The sequence of *mauD* suggests that it may be a disulfide isomerase, which could be required for correct disulfide bond formation, although this protein has never been isolated. As predicted from the sequence of *mauG*, the gene product MauG possesses two *c*-type hemes [26,27]. This enzyme has been shown to catalyze the final three two-electron oxidation reactions during the formation of TTQ from the two Trp residues on the precursor protein of MADH [26–30] (discussed later). The roles of the *mauJ, mauM* and *mauN* are unknown and they are not required for MADH production.

AADH. The aromatic amine utilization (*aau*) gene cluster also possesses several genes [31]. The genes that encode the structural subunits of AADH and the intervening two genes (*aauBEDA*) are similar to