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Review

Tryptophan tryptophylquinone biosynthesis: A radical approach to posttranslational modification ☆

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ABSTRACT

Protein-derived cofactors are formed by irreversible covalent posttranslational modification of amino acid residues. An example is tryptophan tryptophylquinone (TTQ) found in the enzyme methylamine dehydrogenase (MADH). TTQ biosynthesis requires the cross-linking of the indole rings of two Trp residues and the insertion of two oxygen atoms onto adjacent carbons of one of the indole rings. The diheme enzyme MauG catalyzes the completion of TTQ within a precursor protein of MADH. The preMADH substrate contains a single hydroxyl group on one of the tryptophans and no crosslink. MauG catalyzes a six-electron oxidation that completes TTQ assembly and generates fully active MADH. These oxidation reactions proceed via a high valent *bis*-Fe(IV) state in which one heme is present as Fe(IV)=O and the other is Fe(IV) with both axial heme ligands provided by amino acid side chains. The crystal structure of MauG in complex with preMADH revealed that catalysis does not involve direct contact between the hemes of MauG and the protein substrate. Rather it is accomplished through long-range electron transfer, which presumably generates radical intermediates. Kinetic, spectrophotometric, and site-directed mutagenesis studies are beginning to elucidate how the MauG protein controls the reactivity of the hemes and mediates the long range electron/radical transfer required for catalysis. This article is part of a Special Issue entitled: Radical SAM enzymes and Radical Enzymology.

1. Introduction

Tryptophan tryptophylquinone (TTQ) is the protein-derived cofactor of certain amine dehydrogenases [1]. Protein-derived cofactors are catalytic or redox-active centers of proteins that are formed by post-translational modification of one or more amino acid residues [2,3]. TTQ is formed by a post-translational modification of two tryptophan residues of the polypeptide chain. This review will focus on the biosynthesis of TTQ in methylamine dehydrogenase (MADH) from *Paracoccus denitrificans*. In that enzyme, two atoms of oxygen are incorporated into the indole ring of residue βTrp57 and a covalent bond is formed between the indole rings of βTrp57 and βTrp108 (Fig. 1).

MADH catalyzes the oxidative deamination of methylamine to formaldehyde plus ammonia [4] and transfers the electrons derived from the amine substrate to a type 1 copper protein, amicyanin [5,6]. In doing so MADH allows the host organism to use methylamine as a sole source of carbon, nitrogen and energy. Crystal structures have been determined of MADH alone [7], MADH in complex with amicyanin [8], and MADH in complex with amicyanin and cytochrome *c*-551i [9], the electron acceptor of amicyanin in this soluble electron transfer chain. MADH is a heterodimer of two 45 kDa α subunits and two 14 kDa β subunits, the latter each possessing TTQ [7] (Fig. 1). TTQ is critical for both the catalytic and redox properties of MADH as it physically bridges active site chemistry, the oxidative deamination of methylamine, and surface mediated electron transfer to amicyanin.

The biosynthesis of MADH requires not only the post-translational modifications to generate TTQ, but also formation of six disulfide bonds in the β subunit, export of the protein subunits to the periplasm, and assembly of protein subunits. The genes encoding the α and β subunits of MADH are located in the methylamine utilization (*mau*) gene cluster [10]. The *mau* cluster of *P. denitrificans* has 11 genes with a gene order of *mauRFBEDACJGMN* [11]. The α and β subunits of MADH are encoded by *mauB* and *mauA*, respectively, and *mauC* [12] encodes the electron acceptor for MADH, amicyanin. Deletions of either *mauF* [11], *mauD* [13], *mauE* [13] or *mauG* [11] resulted

Abbreviations: MADH, methylamine dehydrogenase; TTQ, tryptophan tryptophylquinone; preMADH, the biosynthetic precursor protein of MADH with incompletely synthesized TTQ; *bis*-Fe(IV) MauG, redox state of MauG with one heme as Fe(IV)=O and the other as Fe(IV); E_m, oxidation-reduction midpoint potential; Compound I, cpd I; Compound ES, cpd ES.

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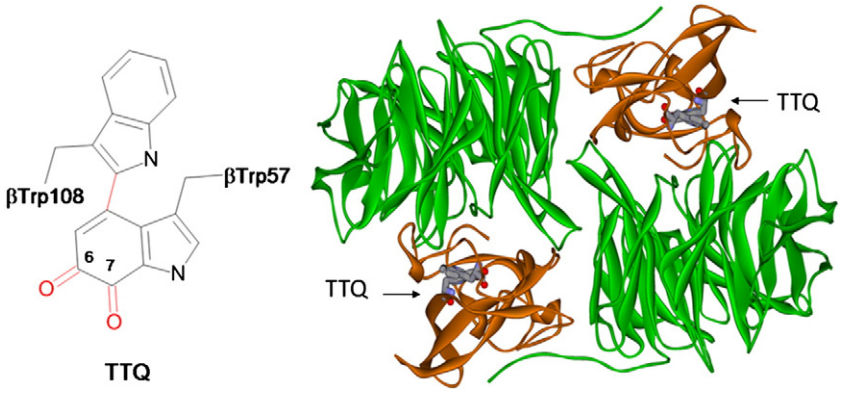

Fig. 1. The protein derived tryptophan tryptophylquinone (TTQ) cofactor of methylamine dehydrogenase (MADH). The structure of TTQ is shown on the left with the posttranslational modifications of residues βTrp57 and βTrp108 colored red. The crystal structure of MADH is shown on the right the α subunits in green and the β subunits in orange. The TTQ of each β subunit is displayed as sticks. The coordinates used for this figure are from PDB ID: 2BBK.

in loss of both MADH activity and the ability of the bacterium to grow on methylamine. In the first three deletions, no MADH protein subunits could be detected in cell extracts. Cells with the *mauG* deletion were lacking in MADH activity and were unable to grow on methylamine, but in this case it was shown by Western Blot analysis that near wild-type levels of the MADH β subunit were expressed [11]. This suggested that MauG might play a role in TTQ formation.

2. The role of MauG in TTQ biosynthesis

A key step in beginning to elucidate the role of MauG in TTQ biosynthesis was the development of a recombinant expression system for MADH. A plasmid which contained the structural genes for MADH as well as *mauFEDG,* the genes required for MADH biosynthesis, was placed in *Rhodobacter sphaeroides* and active recombinant MADH with the correctly synthesized TTQ cofactor was isolated from these cells [14]. To specifically test the role of MauG in TTQ biosynthesis, the *mauG* gene was inactivated in this expression system by site directed mutagenesis. The MADH which was isolated from this altered expression system was inactive and lacked the visible absorption spectrum characteristic of the TTQ cofactor. Analysis of this inactive form of MADH by mass spectrometry revealed that the majority species that was isolated was a biosynthetic intermediate of MADH with incompletely synthesized TTQ containing βTrp57 which was mono-hydroxylated and with no covalent cross-link to residue βTrp108 [15] (Fig. 2). This species was designated preMADH. Incubation of preMADH in vitro with purified MauG and oxidation equivalents provided by molecular oxygen plus an electron donor, or by H_2O_2, resulted in completion of TTQ biosynthesis and formation of active MADH [16]. Thus, while the mechanism by which the first oxygen is inserted into residue βTrp57 is not known, it is known that MauG is required to complete TTQ biosynthesis from that intermediate point. The position of insertion of the second oxygen into βTrp57 was determined by $^{18}O_2$ labeling studies of the MauG-dependent biosynthetic reaction to be the C6 position [17] and it follows that preMADH is hydroxylated exclusively at the C7 position.

3. Physical properties of MauG

When *P. denitrificans* is grown with methylamine as the sole carbon source, the cells are induced to produce large amounts of MADH. However, the MauG protein had never been detected in extracts of these cells. To enable study of MauG, a homologous expression system for expression of this protein was developed in *P. denitrificans* [18]. The MauG isolated from this expression system was shown to be a 42.3 kDa protein which possesses two *c*-type hemes, as was predicted from the gene sequence that contains two CXXCH motifs in which the two Cys residues form covalent thioether linkages to the heme and the His provides an axial ligand [18]. The visible absorption spectra of diferric and diferrous MauG are typical of those of *c*-type cytochromes. The EPR spectrum of fully oxidized MauG reveals that the two ferric hemes are present in a distinct spin state; a high-spin ferric heme that is ligated with a His ligand and a low-spin ferric heme that is six-coordinate with two protein ligands [18]. The crystal structure of the MauG-preMADH complex (Fig. 3) confirmed the presence of a five-coordinate and six-coordinate heme and revealed that the low-spin heme possessed a His–Tyr ligand set [19]. Natural Tyr–His ligation to a *c*-type heme

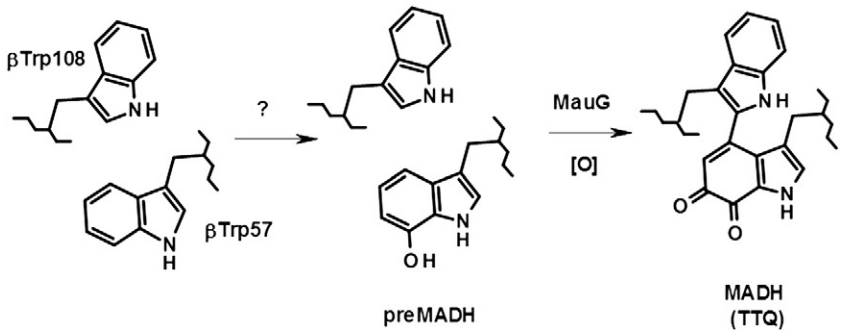

Fig. 2. The role of MauG in TTQ biosynthesis. MauG catalyzes the conversion of monohydroxylated βTrp57 and βTrp108 of preMADH to TTQ. Oxidation equivalents ([O]) may be provided by O_2 plus an electron donor or by H_2O_2.