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Crystallographic investigations of the tryptophan-derived cofactor in the quinoprotein methylamine dehydrogenase

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A model of tryptophan tryptophylquinone (TTQ), recently proposed by McIntire et al. (Science (1991) 252, 817–824) to be the prosthetic group of the quinoprotein methylamine dehydrogenase, has been compared with electron density maps of this dehydrogenase from *Thiobacillus versutus* and *Paracoccus denitrificans*. The comparison shows that the TTQ model can be neatly accommodated, providing strong supportive evidence that TTQ is indeed the cofactor for this group of quinoproteins.

Methylamine dehydrogenase; Quinoprotein; Redox cofactor; Tryptophan tryptophylquinone; Pyrroloquinoline quinone; Amino acid-derived cofactor

1. INTRODUCTION

Methylamine dehydrogenase (EC 1.4.99.3, MADH) catalyzes the oxidative deamination of primary amines [1] and transfers electrons to a *c*-type cytochrome [2] through a mediating blue copper protein, amicyanin [3]. MADH from *Thiobacillus versutus* (TV-MADH) is a hetero-tetramer consisting of two copies of two different subunits, with molecular weights 47.5 kDa for the heavy subunit (H) and 12.9 kDa for the light (L) subunit [4]. MADH from *Paracoccus denitrificans* (PD-MADH) is also an H_2L_2 tetramer with subunit molecular weights of 46.7 kDa and 15.5 kDa for H and L, respectively [5].

One of the most intriguing aspects of the MADH structure is the chemical nature of its redox cofactor, which is located in the L subunit. For more than a decade, MADH has been considered to be a quinoprotein [6], containing covalently-bound pyrroloquinoline quinone (PQQ, Fig. 1a)[7]. However, the X-ray study of TV-MADH had shown that the density for the cofactor in this enzyme was not consistent with PQQ [8].

One of the major obstacles in determining the nature of the cofactor of MADH was lack of knowledge of the nature of the amino acids which were involved in cofactor binding. The chemical sequence of the L subunit of MADH from *Methylobacterium extorquens* AM1 (formerly called *Pseudomonas* AM1) [9], could not identify two residues at positions 55 and 106, suggesting these as being the sites for covalent attachment of the cofactor. This situation has recently improved with the determination of the DNA sequence of the L subunit of MADH from *M. extorquens* AM1 [10], which revealed both residues to be tryptophans. Based on this sequence information and results of mass spectroscopy and NMR experiments on MADH from *Bacterium* W3A1, McIntire et al. recently proposed [11] a new cofactor model for MADH, named tryptophan tryptophylquinone (TTQ, Fig. 1b). In TTQ, the two indole rings are covalently linked between C4(TRP-A) and C2(TRP-B). The indole moiety of TRP-A would have an ortho quinone structure with two carbonyl groups at the C6 and C7 positions. Thus TTQ bears structural similarity to PQQ but clearly is not derived from it.

The universal nature of the TTQ cofactor among MADH's is supported by the similarities among these enzymes. The partial sequence data of PD-MADH so far available, including the chemical sequence of the first 25 residues (unpublished results) and the gene sequence of the last 31 residues [12] show 60% and 95% identity with the AM1 enzyme, respectively. Furthermore, recent resonance Raman spectroscopic studies of MADH's indicate that the enzymes from PD, TV and W3A1 possess the same quinone cofactor [13]. Indeed, the partial gene sequence of the L subunit of PD-MADH [12] has definitely confirmed that position 107

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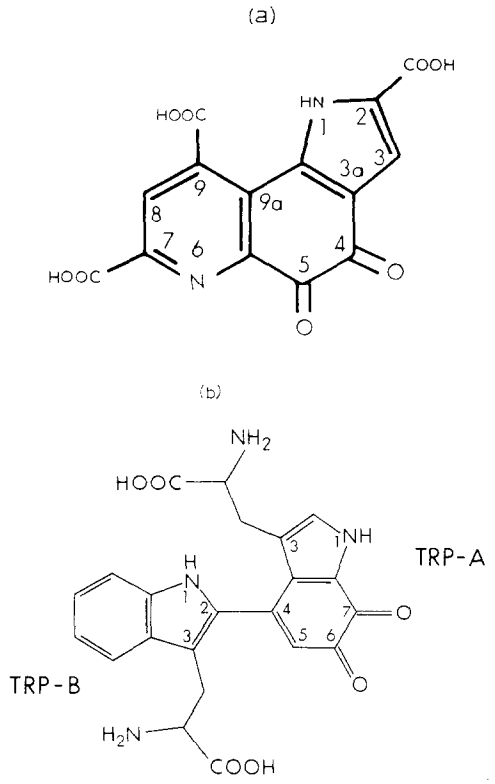

Fig. 1. (a) Pyrroloquinoline quinone (PPQ). (b) Tryptophan tryptophylquinone (TTQ): Tryptophans TRP-A and TRP-B correspond to positions 55 and 106 on the MADH L subunit from *M. extorquens* AM1, or positions 57 and 107 in the TV-MADH 'X-ray sequence'.

is occupied by tryptophan. In order to evaluate the correctness of the new cofactor model, we describe here the results of the fit of the TTQ cofactor into the electron density of both TV- and PD-MADH.

2. MATERIALS AND METHODS

For the comparison of the cofactor with the electron density of TV-MADH, phases obtained from the refined structure [14] were used. In the absence of sequence information this model contains an 'X-ray sequence' based on the electron density distribution, comprising 368 residues for each H subunit and 121 residues for each L subunit. This yielded after refinement a crystallographic *R*-factor of 24.5% for data between 8.0 and 2.2 Å.

During modelling and refinement of the TTQ cofactor, geometric restraints normally used for tryptophan side chains were imposed. In addition, the bond length between atom C4 of residue 57 and C2 of residue 107 was restrained to 1.40 Å, while the C6-O6 and C7-O7 bond lengths of the ortho quinone were set to 1.22 Å. Atoms 57:O6, 57:O7 and 107:C2 were kept in the plane of indole ring 57, whereas atom 57:C4 was restrained to be in the plane of indole ring 107. The manually modelled TTQ cofactor was refined against the diffraction data using the program package TNT [15].

For the comparison of the cofactor with the electron density of PD-MADH a 2.6 Å model was used. This model had been obtained by molecular replacement using the structure of TV-MADH as the search model and subsequently refined to a crystallographic *R*-factor of 24.6%. Details of the structure elucidation will be published elsewhere.

The cofactor was fit in a map, obtained after twofold averaging about the non-crystallographic diad [16]. For the modelling, geometric constraints of tryptophan side chains were imposed on the cofactor. Additionally, the length of bond 57:C4-1O7:C2 was constrained to 1.40 Å and the lengths of bonds C6-O6 and C7-O7 to 1.21 Å.

3. RESULTS AND DISCUSSION

Both TV- and PD-MADH are tetramers, consisting of two HL dimers, related by a molecular two-fold axis. The active site of each HL dimer is close to a narrow channel between the H and L subunits. The quinone cofactor is located in a channel, about 5 Å below the surface of the protein. During previous structural analyses of both TV- and PD-MADH, difficulties had been encountered in fitting the PQQ structure into the electron density at the active sites. In both cases, the density for one of the three carboxylate groups of PQQ appeared to be entirely missing.

In the structure of TV-MADH, PQQ would not fit the electron density. An alternative cofactor model called pro-PQQ, was proposed [8], which had a quinone indole structure to which a glutamic acid was attached, and was linked to the protein at residues 57 and 107. Later, a modified model was discussed, called TGA-proPQQ [17], in which the quinone indole moiety was linked directly to a glutamate and an arginine side chain, which were presumed to be the residues at positions 57 and 107, respectively.

In the averaged 2.6 Å density map of PD-MADH, the electron density corresponding to the cofactor seemed large enough to accommodate PQQ with two discrete linkages to residues 57 and 107, tentatively assigned as serine and arginine, respectively. It proved to be difficult, however, to maintain good geometry for the side chains while forming covalent links to PQQ. Attempts to fit the TGA-proPQQ model into the PD-MADH density were not convincing.

The introduction of TTQ into the active site density of PD-MADH improves the fitting dramatically (Fig. 2). For position 57, the indole-quinone structure fits almost perfectly, with the two carbonyl groups well-defined within the density. The backbone portion of 57 fits into the density without geometrical difficulties which were encountered with a PQQ model. At position 107, the problem of surplus density in the previous effort to fit an arginine at this position is now solved by the second indole ring which is much larger than arginine. The planes of the two indole rings are twisted with respect to each other by about 42°. This is sufficiently large to prevent steric hindrance between the two tryptophan side chains.

For TV-MADH, the placement of tryptophan at positions 57 and 107 also clarifies the interpretation of the electron density. After refinement the TTQ cofactor fits the electron density distribution beautifully (Fig. 3). Omission of the TTQ coordinates followed by several refinement cycles leaves the density virtually unaltered,

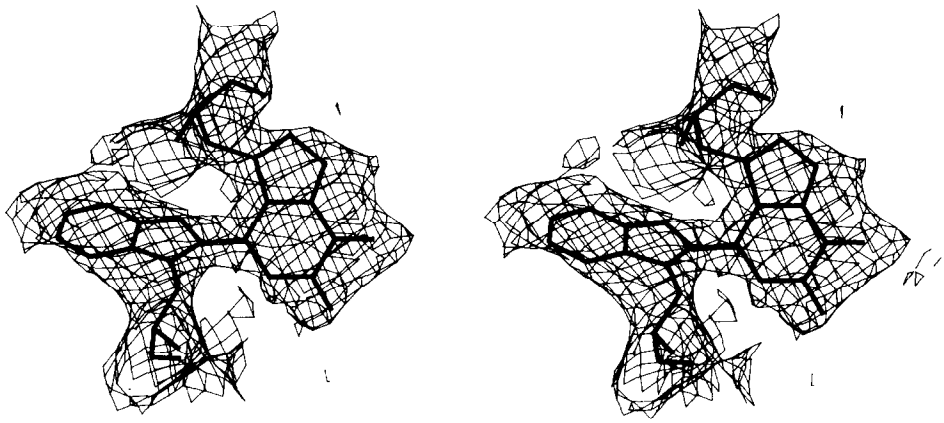

Fig. 2. PD-MADH electron density fitted with the TTQ cofactor. The best fit of the two tryptophans to the density is achieved with a dihedral angle of ~42° between two indole rings.

providing further evidence for the TTQ cofactor structure. An analysis of the hydrogen bonds formed between the TTQ cofactor and surrounding residues (Fig. 4) shows that all atoms of the cofactor which are capable of forming hydrogen bonds indeed do so.

Interestingly the indole-quinone side chain of TRP-57 is quite similar to the previously suggested TGA-proPQQ structure [17]. Ten atoms of the quinone indole ring of the newly refined TTQ cofactor agree within 0.5 Å with the indole ring of the proposed TGA-proPQQ structure. The two carbonyl groups of the TQQ cofactor are pointing into the same active site channel region between the H and L subunits as proposed for the TGA-proPQQ structure. This channel would accommodate the substrate. Also, atom 06 of the modified TRP-57 is close to the binding site for phenylhydrazine which had been identified crystallographically [8].

Using TTQ as the model for the redox cofactor nicely solves initial difficulties encountered during the interpretation of the electron density of both PD- and TV-MADH. In turn, the results obtained from the crystal structures of both enzymes strongly support the new cofactor model. Recent developments indicate that MADH is not unique in having an unusual cofactor previously thought to be PQQ. Janes et al. have provided evidence that the redox cofactor of serum amine oxidase is 6-hydroxydopa (TOPA) [18], instead of PQQ. Similarly, an X-ray study of galactose oxidase has shown that this enzyme contains a tyrosine and a cysteine residue linked through an ether linkage and not PQQ [19]. In this enzyme, the tyrosine is a free radical. Therefore PQQ seems not as universal a redox cofactor for quinoproteins than was originally thought. Since both TOPA and TTQ contain a quinone structure which may serve as the oxido–reduction reaction site, it seems reasonable that the term 'quinoprotein' should have a broader definition, and not be limited to those enzymes containing PQQ as the redox cofactor. This would include other possible organic species with a quinone structure (*ortho* or *para*) as redox cofactor for certain enzymes and these enzymes could all be referred to as 'quinoproteins'.

The proposal of the TQQ cofactor in MADH resolves elegantly the structural and chemical inconsistencies of the previous cofactor models. However,

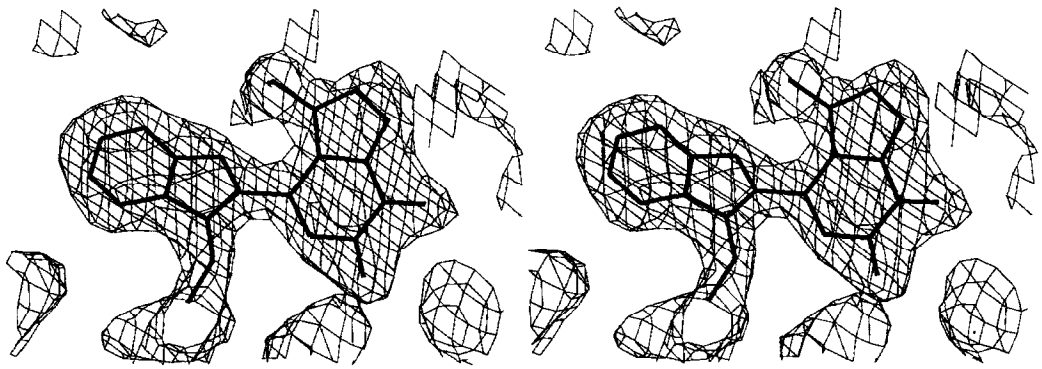

Fig. 3. The refined model and electron density of the TTQ cofactor in TV-MADH. The dihedral angle between the indole rings is about 40°.

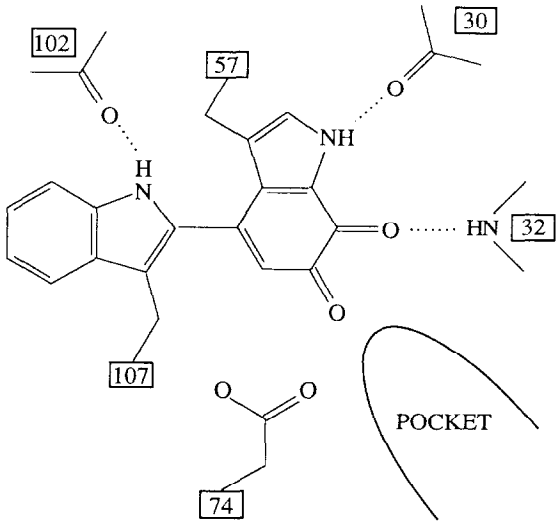

Fig. 4. Schematic drawing of the hydrogen bonds between the TTQ cofactor and surrounding residues in TV-MADH. Hydrogen bonds are formed from backbone carbonyl atoms of residues 30 and 102 and to the backbone amide hydrogen atom of residue 32. Although Asp-74 is within hydrogen bonding distance of atom 06 of the cofactor, actual hydrogen bond formation will depend on both the oxidation state of the cofactor and the protonation state of Asp-74, neither of which is known with certainty.

the new cofactor presents a new set of intriguing and challenging problems, namely, the biosynthesis of the TTQ cofactor. Is the cross-linking and quinolization of the tryptophan groups a self catalytic process or are one or more enzymes involved in the side chain modification? Does the post-translational modification occur soon after polypeptide synthesis or in the mature enzyme? Are one or several modification-responsible enzymes always present in the organism or are they induced by substrate, just as MADH is induced by methylamine? These are some of the new interesting questions to be answered in the amazingly diverse field of recently discovered amino acid-derived cofactors which include TTQ, TOPA and tyrosine free radical.

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