Catalytic mechanism of quinoprotein methanol dehydrogenase: A theoretical and x-ray crystallographic investigation

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The catalytic mechanism of the reductive half reaction of the quinoprotein methanol dehydrogenase (MDH) is believed to proceed either through a hemiketal intermediate or by direct transfer of a hydride ion from the substrate methyl group to the cofactor, pyrroloquinoline quinone (PQQ). A crystal structure of the enzyme-substrate complex of a similar quinoprotein, glucose dehydrogenase, has recently been reported that strongly favors the hydride transfer mechanism in that enzyme. A theoretical analysis and an improved refinement of the 1.9-Å resolution crystal structure of MDH from *Methylophilus methylotrophus* W3A1 in the presence of methanol, reported earlier, indicates that the observed tetrahedral configuration of the C-5 atom of PQQ in that study represents the C-5-reduced form of the cofactor and lends support for a hydride transfer mechanism for MDH.

Methanol and glucose dehydrogenases are two well studied quinoproteins that use 2,7,9-tricarboxypyrroloquinoline quinone (PQQ) as a cofactor (Fig. 1*A*; refs. 1–4). Both enzymes require a divalent cation such as Ca^{2+} for catalytic activity. Crystallographic studies have been reported for both methanol dehydrogenase (MDH) and glucose dehydrogenase (5–12). These crystallographic investigations have not only provided detailed information concerning the PQQ binding site, but they have also established structural frameworks for mechanistic elucidation. Two plausible mechanisms have been proposed. The first one (Fig. 1*B*) involves a nucleophilic addition of the alcohol to the C-5 carbonyl followed by an intramolecular retro-ene reaction (13–16). The second mechanism (Fig. 1*C*) involves a general base initiated hydride transfer from the alcohol to the C-5 carbonyl carbon and subsequent tautomerization of the hydride transfer intermediate to the reduced PQQH2 (15–17). Accurate structural information will be valuable in distinguishing these two proposed mechanisms.

Recently, a high-resolution structure of methanol dehydrogenase from *Methylophilus methylotrophus* W3A1 in a second crystal form has been reported (8). Surprisingly, even when planarity constraints were applied during the structural refinement, the refined structure displayed a C-5 center significantly distorted from planarity. Although the cofactor was assigned to be the semiquinone form of PQQ, this did not provide a satisfactory explanation as to why the C-5 center is tetrahedral, because earlier quantum mechanical studies had demonstrated that the tricyclic ring in the semiquinone form of PQQ is essentially planar in the presence or absence of Ca^{2+} (17). The C-5 methanol adduct could give a tetrahedral C-5 center, but it is not compatible with the electron density map. The tautomeric form of the reduced PQQ, in which the pyrrole H-N hydrogen is moved to the C-5 carbon, does not seem to be plausible owning to a strong intramolecular hydrogen bond interaction between the pyrrole H-N and one of the C-9 carboxylate oxygen atoms. Thus, the observed tetrahedral C-5 remained an unresolved issue. However, if one looks at the second proposed mechanism, the first intermediate is the C-5 reduced form of the reduced PQQ and it does have a tetrahedral C-5 center. Is it possible that the cofactor in the crystal structure is actually the C-5 reduced intermediate? Here, we report a theoretical and crystallographic study aimed at addressing this question.

First, we extended the refinement of the second crystal form of MDH to the full resolution range of the data observed (500–1.9 Å) by using the refinement protocol CNS (18) rather than XPLOR as used previously (8). The x-ray data had been collected from two crystals at room temperature by using Weisenberg geometry (19) at the High Energy Accelerator Research Organization synchrotron facility (Tsukuba, Japan). The CNS refinement resulted in improved agreement of the observed and calculated structure factors and of the geometry, with final R/R_{free}‖ = 0.161/0.190 (previously 0.183/0.209) and root mean square deviations from ideal bond distances/angles of 0.005 Å/1.40° (previously 0.016 Å/1.52°). Electron density maps computed in CNS from the refined structure, but with methanol omitted from the model, showed that the C-5 carbon atom of PQQ remained fully tetrahedral, but that the electron density previously assigned to methanol was altered. The density for the methanol carbon atom was absent, but a spherical peak was present about 0.5 Å displaced from where the methanol oxygen atom had been and may represent a bound water molecule.

Next, we used a computational quantum mechanical approach to examine the structure of the C-5 reduced intermediate. The structure of this intermediate was energy minimized by using a hybrid density functional method at the B3LYP/6-31 + G(d) level of theory (20, 21). The calculations were carried out by using the JAGUAR 4.5 program (Schrodinger, Portland, OR). We examined both the hydroxyl form (C5-OH) and the anion form (C5-O^-). As far as the tricyclic ring is concerned, both structures are essentially the same. Fig. 2 displays the overlap of the calculated structure of the C-5-reduced intermediate and the distorted PQQ as deposited in the PDB file. Apart from the differences in the orientation of

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Abbreviations: MDH, methanol dehydrogenase; PQQ, pyrroloquinoline quinone.

Data deposition: The atomic coordinates and structure factors have been deposited in the Protein Data Bank, www.rcsb.org (PDB ID code 1G72).

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‖$R = \Sigma_h|F_o - F_c|/\Sigma_h|F_o|$, where F_o and F_c are the observed and calculated structure factor amplitudes of reflection h; R_{free} is the test reflection data set, about 10% of the observed structure factors selected randomly for cross validation during crystallographic refinement.

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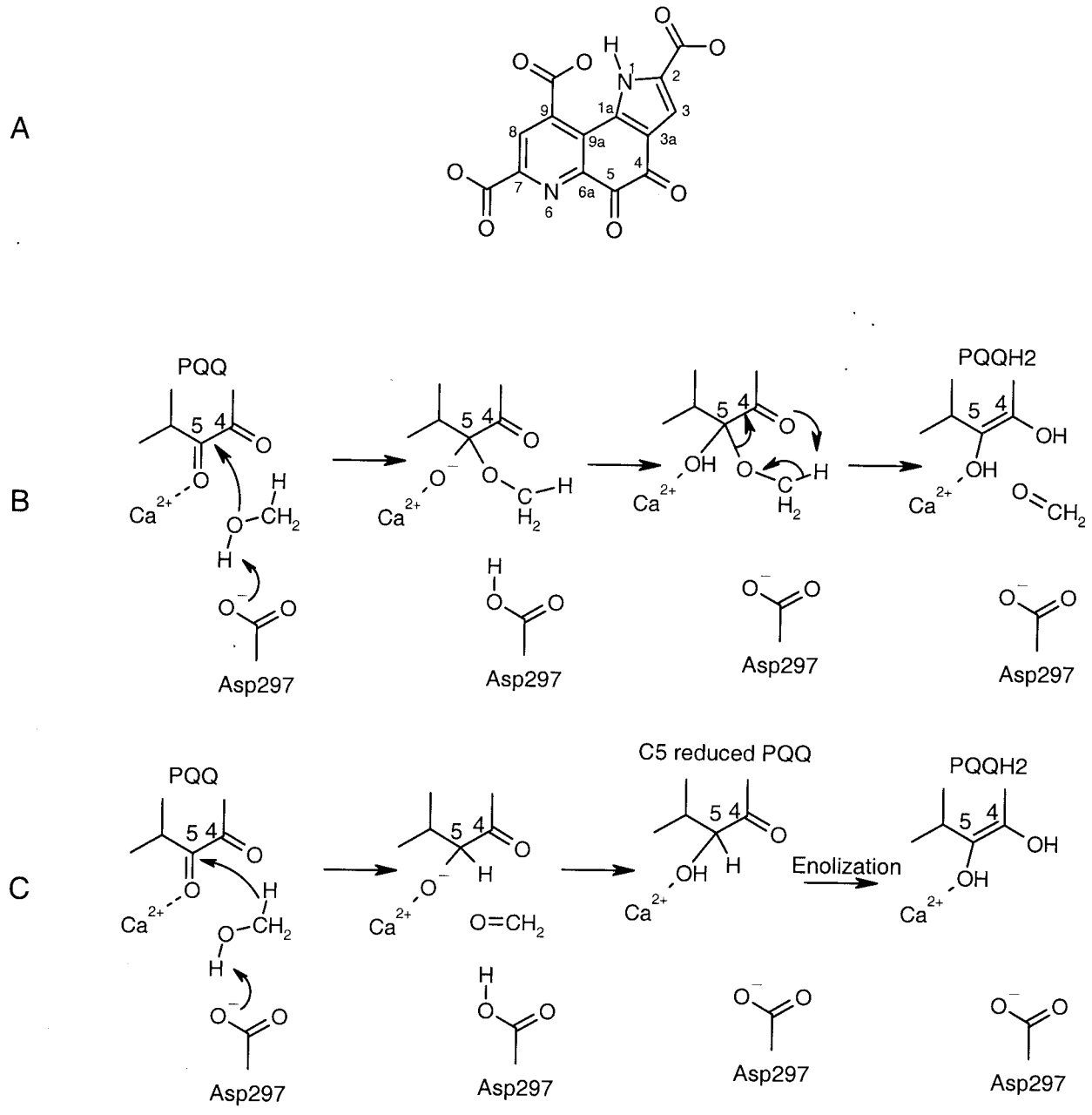

Fig. 1. (*A*) Chemical structure of PQQ with the numbering of the ring atoms indicated. (*B*) Addition-elimination mechanism for oxidation of methanol by PQQ in methanol dehydrogenase involving base catalysis by Asp297. (*C*) Hydride transfer mechanism for methanol oxidation involving a C-5-reduced intermediate of PQQ and base catalysis by Asp297. The last step, enolization, is probably mediated by Asp297

the three carboxylates, the two tricyclic ring structures are essentially identical. The differences in the orientation of the carboxylates are because of the presence of protein side chains in the crystal structure; our calculations were done on the isolated molecule, free of any intermolecular interactions. Inclusion of several active site side chains in our calculation did not change the overall picture. Thus, it is very plausible that the distorted PQQ in the crystal structure of methanol dehydrogenase is actually the C-5-reduced intermediate. This would eliminate the necessity to explain the tetrahedral C-5 center. The immediate implication of this hypothesis is that methanol dehydrogenase follows a hydride transfer mechanism, which is in agreement with the hydride transfer mechanism for glucose dehydrogenase demonstrated recently by Dijkstra and coworkers (11, 12, 22).

The water molecule located above the C-5 atom of PQQ is about 3.0 Å from it and about 3.0 Å from atom OD2 of Asp297, thus apparently forming hydrogen bonds to both atoms (Fig. 3). Although participation of such a weak C-H···O hydrogen bond is unexpected, recent reports have indicated its ubiquitous presence in proteins (24, 25). Indeed, at lower contour levels (slightly below the significance level), the electron density for the water tended to merge with that of PQQ, suggesting such an interaction. However, attempts to model the water as a diatomic molecule covalently bound to C-5, such as a hydroperoxide, were unconvincing. The interaction of the water molecule, located in the relatively hydrophobic environment above the PQQ molecule in MDH, may help stabilize the reduced C-5 tautomer of PQQ in the crystal structure of the enzyme.

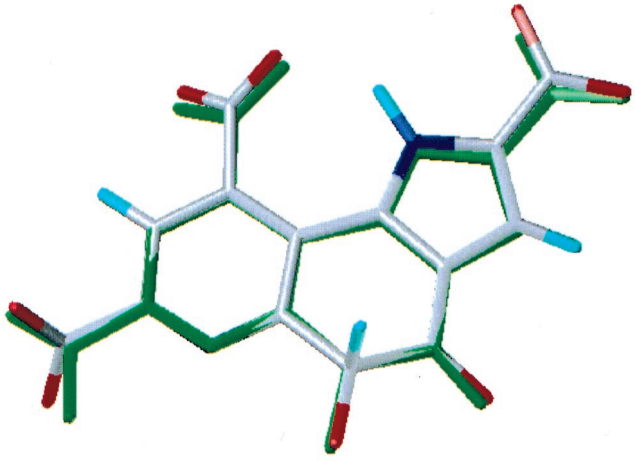

Fig. 2. The overlap of the calculated C-5-reduced PQQ (atom colors: carbon, white; nitrogen, blue; oxygen, red; hydrogen, azure) and the PQQ as refined with CNS (green, hydrogen atoms omitted).

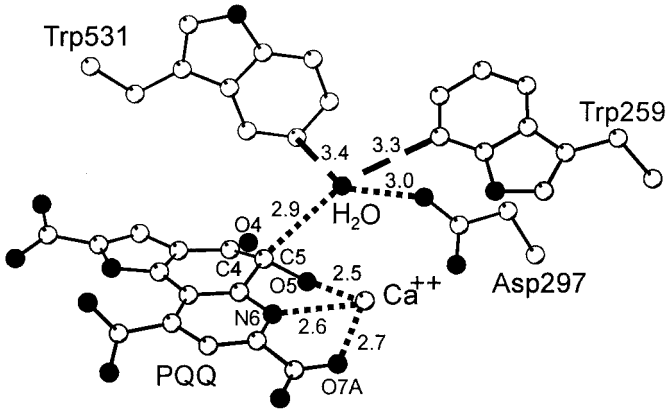

Fig. 3. Environment of the water molecule hydrogen bonded to Asp297 and C-5 of PQQ. Electrostatic and hydrogen bonding interactions are indicated by dotted lines, and van der Waals interactions by dashed lines with distances in Å. This diagram was prepared by using the program MOLSCRIPT (23).

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