REVIEW ARTICLE
Quinoprotein-catalysed reactions

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This review is concerned with the structure and function of the quinoprotein enzymes, sometimes called quinoenzymes. These have prosthetic groups containing quinones, the name thus being analogous to the flavoproteins containing flavin prosthetic groups. Pyrrolo-quinoline quinone (PQQ) is non-covalently attached, whereas tryptophan tryptophylquinone (TTQ), topa-quinone (TPQ) and lysine tyrosylquinone (LTQ) are derived from amino acid residues in the backbone of the enzymes. The mechanisms of the quinoproteins are reviewed and related to their recently determined three-dimensional structures. As expected, the quinone structures in the prosthetic groups play important roles in the mechanisms. A second common feature is the presence of a catalytic base (aspartate) at the active site which initiates the reactions by abstracting a proton from the substrate, and it is likely to be involved in multiple reactions in the mechanism. A third common feature of these enzymes is that the first part of the reaction produces a reduced prosthetic group; this part of the mechanism is fairly well understood. This is followed by an oxidative phase involving electron transfer reactions which remain poorly understood. In both types of dehydrogenase (containing PQQ and TTQ), electrons must pass from the reduced prosthetic group to redox centres in a second recipient protein (or protein domain), whereas in amine oxidases (containing TPQ or LTQ), electrons must be transferred to molecular oxygen by way of a redox-active copper ion in the protein.

INTRODUCTION TO QUINOPROTEINS

Quinoproteins are enzymes whose catalytic mechanisms involve quinone-containing prosthetic groups in their active sites; these may be bound non-covalently or derived from amino acids in the protein backbone of the enzyme (Figure 1). The only non-covalently bound example is pyrrolo-quinoline quinone (PQQ), which is the prosthetic group in a number of bacterial dehydrogenases. Tryptophan tryptophylquinone (TTQ) is derived from two tryptophan residues and occurs in bacterial amine dehydrogenases. Topa-quinone (TPQ) is derived from tyrosine and is the prosthetic group of the copper-containing amine oxidases found in bacteria, yeasts and plants. Lysine tyrosylquinone (LTQ) is also derived from tyrosine together with a lysyl residue, and has been very recently described in a special type of copper-containing amine oxidase, lysyl oxidase. The last few years has seen the identification of these prosthetic groups and determination by X-ray crystallography of examples of each type of enzyme. This review aims to summarize these achievements, to consider the mechanisms proposed for the enzymes and to relate these to each other and to significant features seen in the X-ray structures.

The history of the quinoproteins began in the 1960s with the characterization of the novel prosthetic groups of glucose dehydrogenase by Hauge [1] and methanol dehydrogenase by Anthony and Zatman [2]. More than 10 years later Duine, Frank and co-workers demonstrated that the prosthetic group of methanol dehydrogenase is a quinone structure containing two nitrogen atoms [3,4], and Kennard's group showed it to be PQQ by X-ray diffraction analysis [5]. A number of other bacterial dehydrogenases were subsequently shown, by the groups of

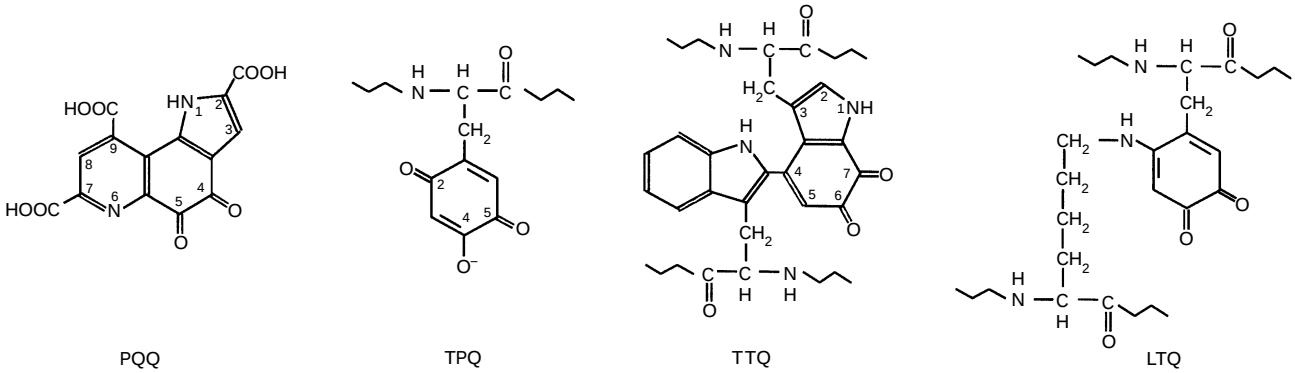


Figure 1 Prosthetic groups of quinoproteins

PQQ is the prosthetic group of some bacterial dehydrogenases. TPQ is the prosthetic group of the copper-containing amine oxidases of bacteria, plants and animals. TTQ is the prosthetic group of bacterial amine dehydrogenases. LTQ is the prosthetic group of lysyl oxidase, a specific copper-containing amine oxidase occurring in animals.

Abbreviations used: LTQ, lysine tyrosylquinone; PQQ, pyrrolo-quinoline quinone; TPQ, topa quinone (6-hydroxyphenylalanine); TTQ, tryptophan tryptophylquinone.

Duine and Frank in Delft and of Ameyama and Adachi in Yamaguchi, to contain PQQ, and the term quinoprotein was coined to include all these proteins [6]. About this time, incorrect identification of PQQ as the prosthetic group of many other enzymes led to considerable confusion, which has now been resolved with the determination of the structure of TTQ by McIntire and his colleagues [7], and of TPQ [8] and LTQ [8a] by Klinman, Dooley and their colleagues.

THE PQQ-CONTAINING DEHYDROGENASES

The only enzymes containing PQQ are bacterial enzymes in which this prosthetic group is tightly, but not covalently, bound; the best known of these quinoproteins catalyse the oxidation of alcohols and glucose in the periplasm of bacteria. They are usually assayed with artificial electron acceptors (A) such as phenazine ethosulphate, when the following reaction is catalysed:

$$R_1R_2CHOH + A \rightarrow R_1R_2C{=}O + AH_2$$

The physiological electron acceptor is a soluble cytochrome *c* in the case of methanol dehydrogenase and some ethanol dehydrogenases; it is protein-bound haem *c* in the quinohaemoprotein alcohol dehydrogenases, and ubiquinone in the membrane-bound glucose dehydrogenase. These enzymes all have a bivalent cation at the active site, and their catalytic subunits are very similar, as indicated by modelling the alcohol dehydrogenase and glucose dehydrogenase sequences on to the co-ordinates of the methanol dehydrogenase of *Methylobacterium extorquens* [9,10].

Methanol dehydrogenase and its prosthetic group (PQQ)

This enzyme catalyses the oxidation of methanol to formaldehyde in the periplasm of methylotrophic bacteria (for general reviews see [11–15], for reviews of relevant electron transport systems see [16,17], and for a review of the biosynthesis of the dehydrogenase and associated electron transport components see [18]). It has an $\alpha_2\beta_2$ tetrameric structure; the α-subunit containing the PQQ has a molecular mass of 66 kDa, and the β-subunit is very small (8.5 kDa) and was not discovered until 25 years after the first description of the enzyme [19]. The subunits cannot be reversibly dissociated and no function has been ascribed to the small subunit. Each α-subunit contains a calcium ion, which is essential for maintaining the PQQ in its active configuration in the active site [20–22].

The prosthetic group (Figure 1) was first isolated, purified and characterized by Anthony and Zatman [2], the X-ray structure of an acetone adduct was determined by Kennard and her colleagues [5], and an extensive chemical characterization was achieved by Frank and Duine and their co-workers (summarized in [12–14]). The pH-dependence of the midpoint redox potential of the $PQQ/PQQH_2$ couple (+90 mV at pH 7.0) indicates that it acts as a $2e^-/2H^+$ redox carrier [23]. Resonance Raman spectroscopy of the isolated PQQ and other quinones, their derivatives and quinoproteins has been extensively reviewed by Dooley and Brown [24,25], and methods for the foolproof measurement of PQQ were reviewed by Klinman and Mu [26].

X-ray structure of methanol dehydrogenase

This is the only PQQ-containing enzyme for which a structure is available [21,22,27–30], the highest resolution structure (1.94 Å) being that of the enzyme from *Methylobacterium extorquens* (the numbering system for amino acids is for this enzyme) [22]. It is an $\alpha_2\beta_2$ tetramer structure, with the small β-subunits folding around the surface of the α-subunits. The α-subunit is a superbarrel made up of eight β-sheets arranged with radial symmetry

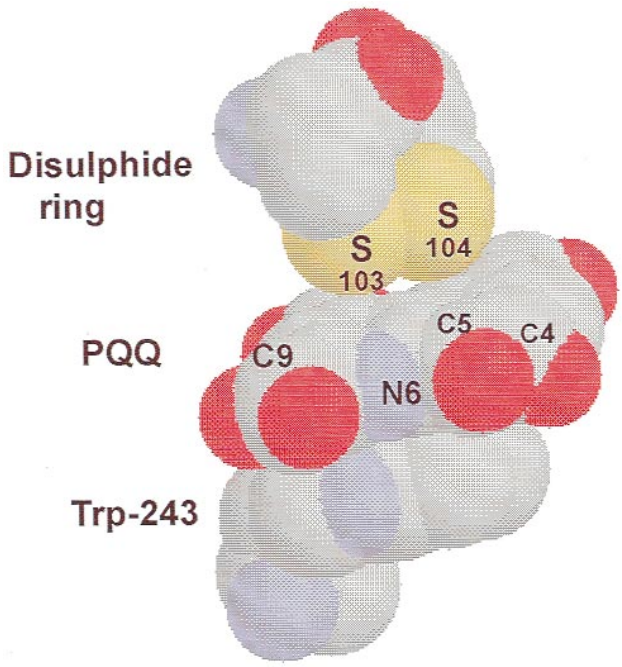


Figure 2 Novel disulphide ring in the active site of methanol dehydrogenase

The ring is formed by disulphide bond formation between adjacent cysteine residues. The PQQ is 'sandwiched' between this ring and the tryptophan that forms the floor of the active-site chamber. The calcium ion (not shown) is co-ordinated between the C-9 carboxylate, the N-6 of the PQQ ring and the carbonyl oxygen at C-5. The oxygen of the C-4 carbonyl is clearly out of the plane of the ring. The full structure is given in [22].

(the 'propeller fold') and held together by novel tryptophan docking motifs [22,30]. The PQQ is buried in the interior of the superbarrel within a chamber that communicates with the exterior through a shallow hydrophobic funnel-shaped depression in the surface. The floor of the chamber is formed by the plane of a tryptophan residue, the ceiling being formed by a novel ring structure arising from a disulphide bridge between adjacent cysteine residues joined by a novel non-planar peptide bond (Figures 2 and 3). In addition, the PQQ is bonded by many equatorial reactions as shown in Figure 4, which also indicates the co-ordination of the calcium ion to the PQQ and the protein. The calcium is co-ordinated to Glu-177 (both carboxylate oxygen atoms) and Asn-261, as well as N-6, the C-5 quinone oxygen and the C-7 carboxyl group of PQQ. Figures 3 and 4 also show a likely active-site base (Asp-303) and indicate that the C-4 carbonyl oxygen atom is clearly out of the plane of the ring, perhaps consistent with the semiquinone state of the prosthetic group in this enzyme [20,22,31].

Reductive half-reaction of methanol dehydrogenase

The reaction mechanism of methanol dehydrogenase has been more difficult to elucidate by kinetic and spectroscopic investigations than those of the other types of quinoprotein. This is because the isolated enzyme contains PQQ in the fully reduced form or as the semiquinone, and so addition of substrate (a two-electron donor) does not lead to its reduction; furthermore, 'endogenous substrate' immediately reduces any oxidized enzyme produced during experiments. Finally, the enzyme becomes inactivated when oxidized with artificial electron acceptors [11,13,14,32]. It catalyses a Ping-Pong reaction, consistent with

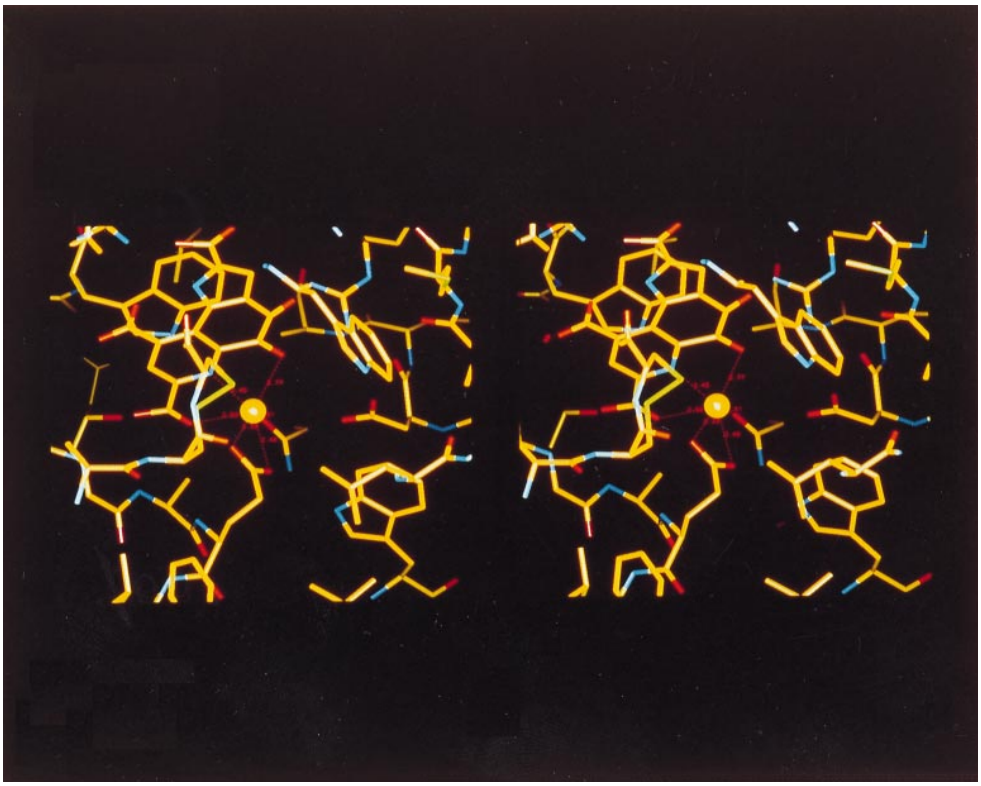


Figure 3 Stereo view of the active site of methanol dehydrogenase centred on the likely substrate-binding site

The PQQ, sandwiched between Trp-243 and the novel ring structure formed by the Cys-103–Cys-104 disulphide bridge, can be seen at the top left. The Ca^{2+} is shown as a yellow sphere ligated by O-5, N-6 and O-7 of PQQ and the side chains of Glu-177 and Asn-261. It was suggested that the proposed active-site base is Asp-303, which is shown at centre right. Reproduced with permission from [22].

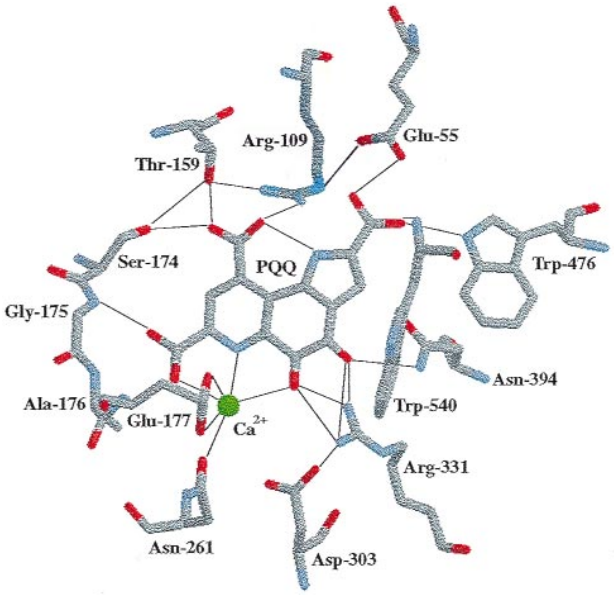


Figure 4 Co-ordination of the calcium ion and PQQ at the active site of methanol dehydrogenase

In addition to the equatorial interactions shown, PQQ is sandwiched between the indole ring of Trp-243 and the disulphide ring structure shown in Figures 2 and 3. The full structure is given in [22].

reduction of PQQ by substrate and release of product, followed by two sequential single-electron transfers to the cytochrome c_L, during which the $PQQH_2$ is oxidized back to the quinone by way of the free radical semiquinone [33–35]. The rate-limiting step is the conversion of the oxidized complex containing the substrate into the reduced enzyme plus product, and is the only step requiring the activator ammonia.

The C-5 carbonyl of isolated PQQ is very reactive towards nucleophilic reagents, adducts being formed with methanol, aldehydes, ketones, urea, cyanide, ammonia and amines [36,37], and this has encouraged the assumption that a covalent PQQ–substrate complex may be important in the reaction mechanism. The reaction of MDH with cyclopropanol gave a C-5 propanal adduct, indicating that the mechanism consists of proton abstraction by a base at the active site, followed by rearrangement of the cyclopropoxy anion into a ring-opened carbanion, and attack of this on the electrophilic C-5 of PQQ [32,38]. It was concluded that during oxidation of methanol a similar proton abstraction must occur, followed by formation of a carbon–oxygen bond to give a hemiketal intermediate. The only direct evidence for this point is the slight change in the spectrum of a possible intermediate seen during reaction with deuterated methanol [34,35]. No evidence was obtained for a covalent intermediate in studies with the barium-containing enzyme, which has some advantages for study of the mechanism of methanol dehydrogenase [39].

In the structure of the enzyme from *Methylophilus* W3A1, a solvent molecule is seen near the O-5 of PQQ. It is suggested that the hydroxy group of methanol could form hydrogen bonds to

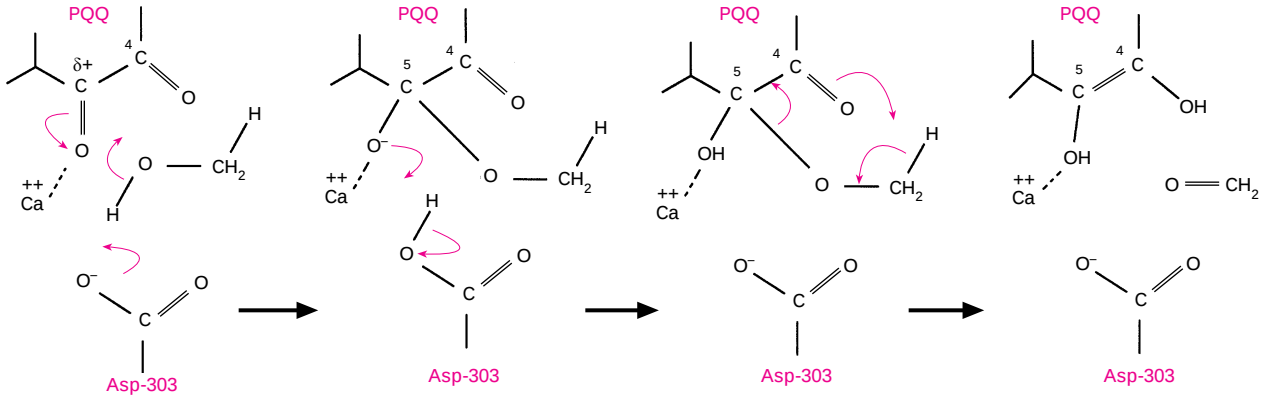


Figure 5 Reaction mechanism for methanol dehydrogenase involving a hemiketal intermediate

Proton abstraction by the base leads to an oxyanion form of the substrate which attacks the electrophilic C-5, giving the hemiketal intermediate from which the methyl proton is abstracted; this in turn gives the quinone and product formaldehyde.

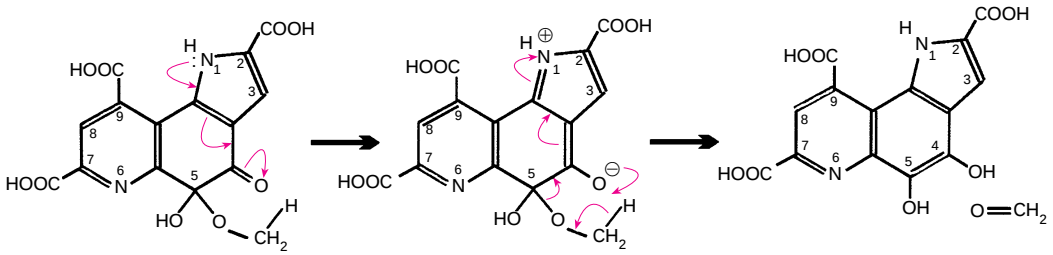


Figure 6 Possible involvement of the pyrrole nitrogen in the reaction mechanism of methanol dehydrogenase

This is a modification of the mechanism shown in Figure 5. In this variation the difficult proton abstraction from the methyl group is facilitated by the ionization of the C-4 carbonyl oxygen, which is made possible by the presence of the pyrrole nitrogen atom.

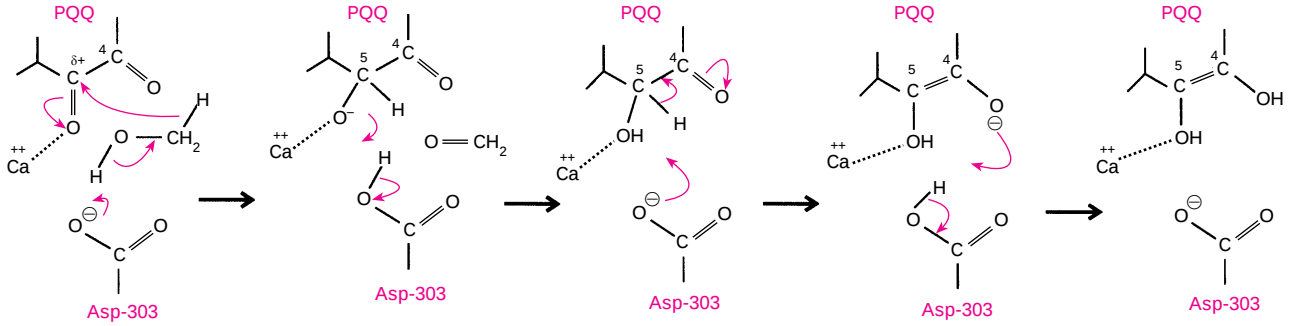


Figure 7 Reaction mechanism for methanol dehydrogenase involving hydride transfer

This mechanism is suggested as an alternative to that shown in Figure 5. The key difference is that there is no covalent bonding of substrate. In this case the initial proton abstraction is the same, but the electrophilic C-5 is involved directly in removal of the methyl hydrogen as a hydride. The active-site base (Asp-303) acts twice in the mechanism.

both O-5 and Asp-303 in the same way as this solvent molecule, and that the substrate methyl group might be accommodated by a hydrophobic cavity, bounded by two tryptophans, a leucine and the disulphide ring [30]. It is probable that Asp-303 (Figures 3 and 4) provides the catalytic base which initiates the reaction by abstraction of a proton from the alcohol substrate (Figures 5–7). In these mechanisms the Ca^{2+} ion is given a role in addition to a structural role in maintaining PQQ in an active configuration; it is proposed that the Ca^{2+} acts as a Lewis acid by way of its co-ordination to the C-5 carbonyl oxygen of PQQ, thus providing the electrophilic C-5 for attack by an oxyanion or hydride [15].

It is also possible that the Ca^{2+} ion co-ordinates to the substrate oxygen atom. The role of Ca^{2+} in the mechanism has been given support by a study of a Sr^{2+}-containing methanol dehydrogenase produced by growing bacteria in a high concentration of Sr^{2+} [40], and by investigations using an active enzyme containing Ba^{2+} instead of Ca^{2+} [39]. This is the first example of an enzyme in which barium plays an active catalytic role; the modified enzyme has a relatively low affinity for methanol (K_m 3.4 mM instead of 10 μM) and for its activator ammonia, but its activation energy is half (and its V_{max} twice) that of the normal Ca^{2+}-containing enzyme. We have suggested that this may be due to a

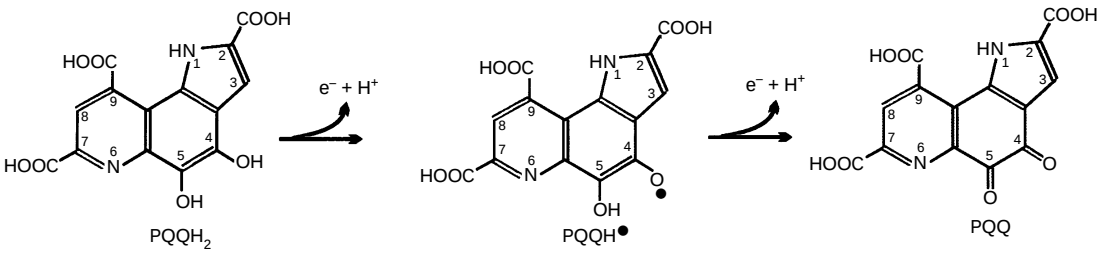


Figure 8 Oxidative half-reaction of methanol dehydrogenase

The free-radical semiquinone (PQQH•) is indicated with the unpaired electron on the C-4 carbonyl oxygen, which is perhaps consistent with the fact that this oxygen is seen to be out of the plane of the rest of the PQQ (see Figure 2). The electron acceptor is either a dye such as phenazine ethosulphate or the natural electron acceptor cytochrome c_L.

change in conformation at the active site, leading to a decrease in the free energy of binding and hence to a decrease in activation energy [39].

In the mechanism shown in Figure 5 the oxyanion produced by proton abstraction attacks the electrophilic C-5, leading to formation of the proposed hemiketal intermediate. The subsequent reduction of the PQQ with release of product aldehyde is likely to be facilitated by prior ionization of the hemiketal complex, which might involve the pyrrole N atom (Figure 6). An alternative mechanism (Figure 7) is a simple acid/base-catalysed hydride transfer in which Asp-303 again provides the base and Ca^{2+} again acts as a Lewis acid.

The large deuterium isotope effect (approx. 6) observed during the reductive phase of the reaction is consistent with either mechanism; in both cases the step affected will be the breaking of the C–H bond, and it is this step that is affected by the activator ammonia [34,39]. Ammonia (the free base) is required as activator when the enzyme is assayed with artificial electron acceptors, but not usually when it is assayed with cytochrome [11,14,34,35,41]. This activation is confined to the step involving hyrogen transfer from the substrate to PQQ, but its mechanism of action is not known [34,35,39]. An obvious possibility would involve formation of a covalent iminoquinone adduct, and one of the first mechanisms proposed for this enzyme suggested that methanol binds as a methoxy group to the same carbon atom (C-4) as the ammonia and is then released as the aldehyde during reduction of the PQQ [13,42]. Although ammonia is able to form an adduct at the C-5 position of isolated PQQ [36,37,43], there is no convincing evidence that this occurs during the enzyme reaction. No nitrogen-containing adduct of PQQ has been isolated from the enzyme; added ammonia is easily removed by gel filtration to give inactive enzyme, which then requires added ammonia for activity. In the reaction with cytochrome this activator is not always required, and some alcohol dehydrogenases which are likely to have essentially similar mechanisms (see below) do not require ammonia as activator.

Oxidative half-reaction of methanol dehydrogenase

This involves two separate single-electron transfer steps to cytochrome c_L, which is a novel class of large acidic *c*-type cytochromes [16,44]. Cytochrome c_L contains five α-helices, the central three of which envelop the haem group and correspond to analogous helices in most other *c*-type cytochromes [45].

There is considerable evidence that the interaction between the dehydrogenase and cytochrome c_L is electrostatic in nature [16,35], and chemical modification studies have indicated that the interaction is by way of a small number of lysyl residues on the dehydrogenase and carboxylates on the cytochrome [46,47]. However, hydrophobic interactions might also be important, as indicated by the X-ray structure of the dehydrogenase. This shows that the PQQ is buried within an internal chamber which communicates with the exterior of the protein by way of a hydrophobic funnel in the surface, which is perhaps the most likely place for interaction with the cytochrome [15,22,41]. A study of the initial 'docking' interaction confirmed the role of electrostatic interactions but, surprisingly, showed that this interaction is not inhibited by 50 μM EDTA, which is sufficient to inhibit the overall electron transfer process [48]. It was therefore suggested that EDTA inhibits by binding to nearby lysyl residues, thus preventing movement of the 'docked' cytochrome to its optimal position for electron transfer, which probably involves interaction with the hydrophobic funnel in the surface of the dehydrogenase [48]. An extensive kinetic investigation of the interaction of the two proteins has led to a similar conclusion [49].

It is reasonable to assume that electron transfer from the quinol form of PQQ to the cytochrome electron acceptor occurs in two single-electron transfer steps, with the semiquinone form of PQQ being produced after the first of these transfers (Figure 8). The protons are released from the reduced PQQ into the periplasmic space, thus contributing to the protonmotive force [17,50]. It was once thought that an intermediary in this process was the novel disulphide bridge between adjacent cysteines in the active site; this novel structure is very readily reduced with Cleland's reagent (dithiothreitol), yielding enzyme that is inactive with cytochrome but active with phenazine ethosulphate [28]. However, the activity with phenazine ethosulphate occurs because the two thiols are rapidly oxidized by this reagent back to the original disulphide; furthermore, no free thiols were ever detected during the reaction cycle, and reaction with iodoacetate led to active enzyme containing carboxymethylated cysteine residues that could no longer take part in oxidation/reduction reactions [31]. This type of disulphide ring structure has not been observed previously in an active enzyme, and its rarity would suggest some special biological function. It is not present in the quinoprotein glucose dehydrogenase, in which electrons are transferred to membrane ubiquinone from the quinol $PQQH_2$ and in which the semiquinone free radical is unlikely to be involved as a stable intermediate. It has been suggested, therefore, that this novel structure might function in stabilization or protection from solvent at the entrance to the active site of the free-radical PQQ semiquinone in methanol dehydrogenase [31].

PQQ-containing dehydrogenases for alcohols and glucose

There are three types of PQQ-containing alcohol dehydrogenases that are distinct from methanol dehydrogenase [51], but they all

contain a calcium ion and the reductive parts of their mechanisms are likely to be similar. The first type, such as that in *Pseudomonas aeruginosa*, is almost identical with methanol dehydrogenase, except for its substrate specificity [52]. The other types are quinohaemoproteins, having an in-built electron acceptor in the form of haem *c*.

The quinohaemoprotein alcohol dehydrogenase from acetic acid bacteria is membrane-bound and contains three types of subunit, but no subunit equivalent to the small β-subunit of methanol dehydrogenase [51,53]. The primary sequence of the catalytic subunit shows an N-terminal region (600 residues) with an additional C-terminal extension containing a haem-binding site [54,55]. In the N-terminal region there is 31 % identity with the sequence of methanol dehydrogenase, and it was possible to model the structure using the co-ordinates of methanol dehydrogenase [9]. Although the model predicts considerable differences in the external loops, particularly those involved in the formation of the shallow funnel leading to the active site, the active-site region was highly conserved, including the tryptophan and the disulphide ring on opposite sides of the plane of the PQQ, and also most of the equatorial co-ordinations to the PQQ (Figures 2–4). Especially important with respect to the mechanism is the conservation of the active-site base (Asp-303 in methanol dehydrogenase) and all the co-ordinations to the calcium ion. This suggests that the mechanism of this alcohol dehydrogenase is essentially similar to that of methanol dehydrogenase. Comparison of the protein sequence of the soluble quinohaemoprotein ethanol dehydrogenase from *Comomonas testosteroni* with that of methanol dehydrogenase leads to a similar conclusion for that enzyme [56].

There are two completely different types of PQQ-containing glucose dehydrogenases in bacteria [13,51,53]. Little is known about the structure or mechanism of the soluble (periplasmic) enzyme, which will not be considered further. The membrane-bound glucose dehydrogenase catalyses the oxidation of the pyranose form of D-glucose and other monosaccharides to the lactone. The reaction occurs in the periplasm, and the electron acceptor is ubiquinone in the membrane [57,58]. The enzyme is an intrinsic monomeric membrane protein (molecular mass $\sim$ 87 kDa) for which a bivalent cation is probably necessary for activity. The N-terminal region (residues 1–154) forms a membrane anchor with five transmembrane segments, and this region is likely to contain the ubiquinone-binding site [59], which is not very similar to that in NADH dehydrogenase in mitochondria [60]. The remaining periplasmic region (residues 155–796) shows 26 % sequence identity with the α-subunit of methanol dehydrogenase, and it has been possible to model its structure using the co-ordinates of methanol dehydrogenase [10]. In the model structure, the novel disulphide ring is replaced by a histidine residue which maintains the position of PQQ in the active site, consistent with the previous demonstration that a histidine residue is essential for binding of PQQ [61]. There are fewer equatorial interactions between the protein and PQQ, perhaps explaining why it is possible to effect the reversible dissociation of PQQ from the glucose dehydrogenase, but not from methanol dehydrogenase [51,62]. The ligation of calcium is similar, suggesting that the calcium plays a similar role in the two enzymes, i.e. that of a Lewis acid through co-ordination to the C-5 carbonyl oxygen, thus providing the electrophilic C-5 of PQQ. The proposed active-site base is conserved, suggesting that the reaction is initiated by abstraction of a proton from the anomeric hydroxy group of the pyranose ring. This would be followed by attack by the resulting oxyanion to form a hemiketal intermediate, or attack by a hydride from the glucose oxyanion leading directly to formation of the lactone and the quinol form of PQQ. That the mechanism might be different, however, is indicated by the fact that Mg^{2+} can replace Ca^{2+} [62,63], which is not possible in methanol dehydrogenase [20]. A previous suggestion [61] that the reaction with glucose is initiated by proton abstraction by a cysteine residue is unlikely to be correct, because there is no cysteine residue within the appropriate region of the active site and all cysteines are involved in disulphide bond formation.

The oxidative half-reaction is likely to be completely different from that in the dehydrogenases for methanol and ethanol. In those enzymes there must be two single-electron transfers to haem *c*, whereas in glucose dehydrogenase two hydrogen atoms must be transferred to the acceptor ubiquinone. It is not necessary, therefore, for a stable semiquinone to be formed, and indeed no semiquinone has ever been observed. The active-site funnel is not hydrophobic, and there is no suggestion from the model structure or from the primary sequence that there is a hydrophobic region of the protein that could interact with the membrane except for the N-terminal transmembrane segments.

TTQ-CONTAINING AMINE DEHYDROGENASES

These enzymes catalyse the oxidative deamination of primary amines to the aldehyde, ammonia and water in the presence of an electron acceptor, which is usually a specific blue copper protein. The dye phenazine methosulphate or ethosulphate (A) is usually used as a convenient artificial electron acceptor in the reaction:

$$RCH_2NH_2 + H_2O + A \rightarrow RCHO + AH_2 + NH_3$$

The first of these enzymes to be described was the methylamine dehydrogenase responsible for methylamine oxidation in the same species of methylotrophic bacteria in which the PQQ-containing methanol dehydrogenase was first discovered; it was first described, and its prosthetic group (TTQ) characterized, by Eady and Large around 1970 [64,65]. It is a periplasmic enzyme and usually uses a blue copper protein called amicyanin as electron acceptor, the reduction of which leads to release of protons into the periplasm, thus contributing to the protonmotive force and hence to ATP synthesis [17,50,66]. Methylamine dehydrogenase has been characterized in detail, and shown to be similar in a number of different methylotrophic bacteria [64,65,67–71]. Davidson has published an outstanding review of the mechanistic and historical aspects of this enzyme [72].

Prosthetic group of methylamine dehydrogenase

When methylamine dehydrogenase was first described, its absorption spectra in the presence and absence of substrate, its sensitivity to carbonyl reagents and its probable Schiff-base formation suggested that a pyridoxal derivative might be acting in a novel fashion as the prosthetic group [65]. This group was subsequently claimed to be a covalently attached form of PQQ, but this possibility was excluded by MS of a derivative of the isolated prosthetic-group peptide [70], and by analysis of X-ray data (at 2.25 Å) obtained for the whole enzyme which suggested a precursor form of PQQ (pro-PQQ) [73]. The structure of the prosthetic group (TTQ; Figure 1) was finally solved by McIntire and his colleagues by an extensive rigorous analysis of the semicarbazide derivative by ^{13}C-NMR and MS [7,74]. Their proposed structure was consistent with the sequence of the gene for the light subunit of methylamine dehydrogenase, indicating that the prosthetic group arose by post-translational modification

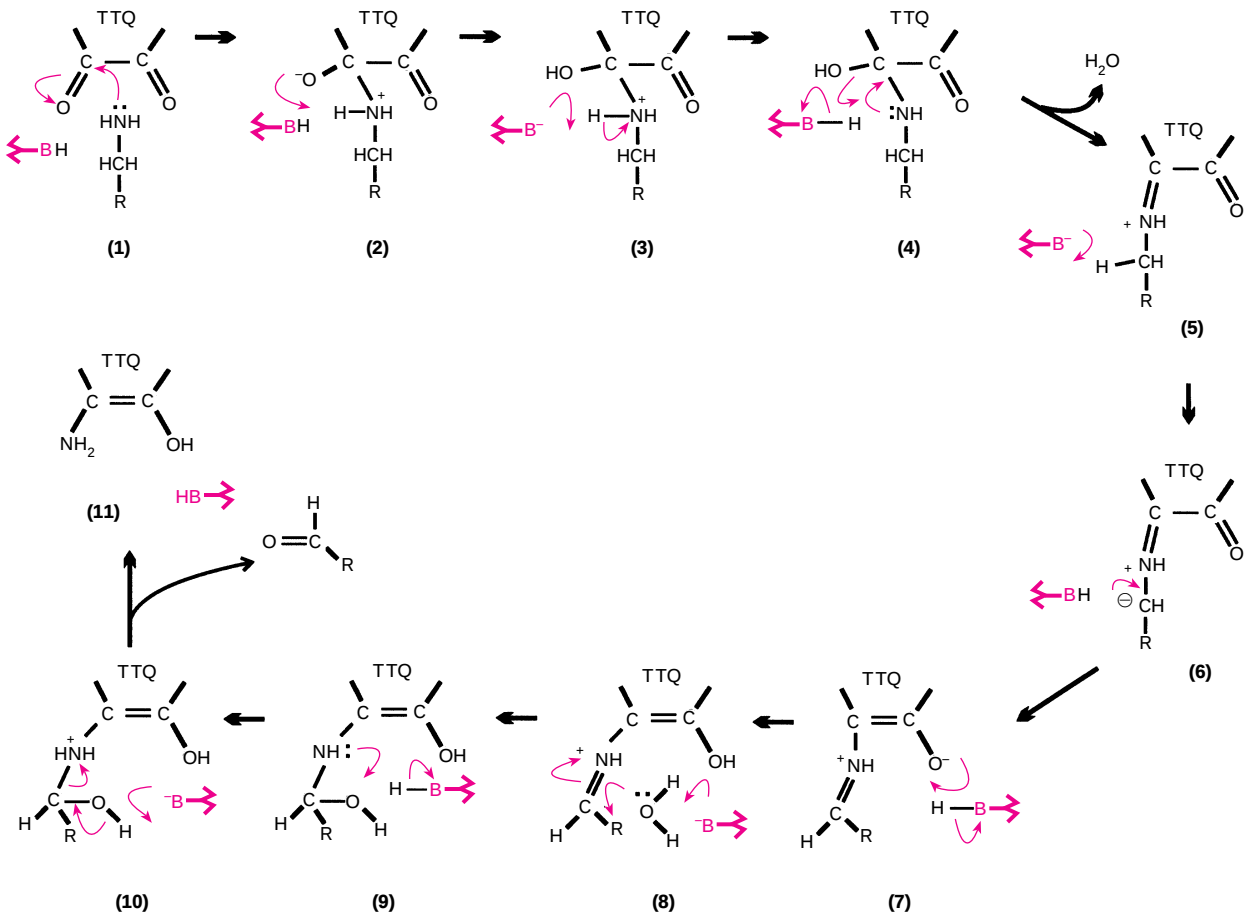


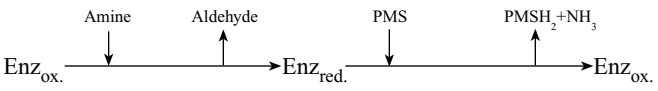
Figure 9 Reductive half-reaction catalysed by amine dehydrogenase

The initial nucleophilic attack by amine substrate gives the carbinolamine (**3**), which loses water to give the iminoquinone Schiff base (**5**). A key feature of this mechanism is the subsequent abstraction by the active-site base ($-B^-$) of a proton from the α-carbon atom of the amine substrate to give the carbanion intermediate (**6**). This leads to reduction of the TTQ ring system with production of the product Schiff base (**7**), which is hydrolysed to the aldehyde product and the aminoquinol form of the enzyme. This Figure shows a base involved in a total of five separate steps in the overall sequence; this may be the same base, but a second base (or acid) may be involved.

of two tryptophans [75]. The structure proposed by McIntire was subsequently shown to fit the X-ray data for methylamine dehydrogenase [76], and resonance Raman spectroscopy has confirmed the same structure in a number of other methylamine dehydrogenases [24,25,77,78].

Reductive half-reaction of methylamine dehydrogenase

Steady-state kinetic studies have demonstrated a Ping-Pong mechanism in which the aldehyde product is released before reaction with phenazine methosulphate (PMS), which oxidizes the enzyme with the concomitant release of ammonia. This evidence, together with measurements of absorption spectra, led to the following postulated reaction sequence [65]:



The proposal that the prosthetic group is an orthoquinone similar to PQQ led to a rationalization of the kinetic and spectral data, leading to the suggestion that the reaction proceeds by initial Schiff-base formation and subsequent production of an aminoquinol [79]. These intermediates have now been confirmed in a wide range of studies involving stopped-flow kinetics and modification with inhibitors and substrates, which have led to the mechanistic proposals described in Figure 8 (see reviews by Davidson and co-workers [72,80] for a full description and analysis of this work, together with methods for determination of the intermediates). The deuterium kinetic isotope effect of 3, measured in steady-state kinetic studies, implies a mechanism involving rate-limiting abstraction of a methyl proton [81]. Subsequent stopped-flow kinetics confirmed this suggestion and demonstrated the presence of two kinetically significant intermediates, a relatively fast transition due to reduction of TTQ by substrate and a slower transition due to release of the aldehyde product [82]. In these experiments, and in similar experiments using the aromatic amine dehydrogenase, an exceptionally large primary deuterium isotope effect was observed [83–85]. The origin of this is unknown, but it is similar to that observed in the proton abstraction step in the TPQ-containing amine oxidase, which was attributed to the phenomenon of quantum-mechanistic tunnelling [86].

Benzylamines are not full substrates of methylamine dehydrogenase, but are competitive inhibitors able to reduce TTQ; analysis of Hammett plots for these reactions led to the proposal

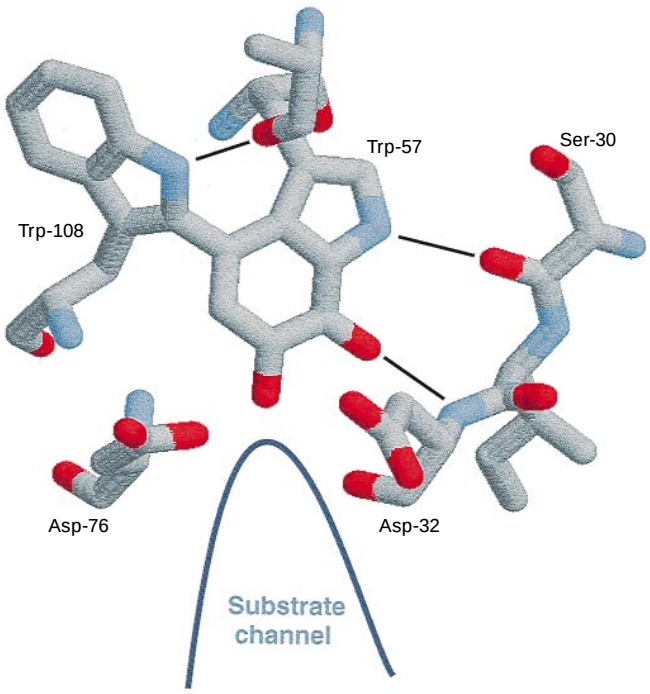


Figure 10 TTQ in the active site of methylamine dehydrogenase from *Paracoccus denitrificans*

The hydrogen bonds to the TTQ are shown together with Asp-76, which is likely to be the active-site base. The full structure of the enzyme is described in [76,93,101].

that a key intermediate in the reductive half-reaction is the carbanion (intermediate **6** in Figure 9) [87]. The involvement of a carbanion in the mechanism is supported by the results of a detailed investigation of a second TTQ-containing enzyme, an aromatic amine dehydrogenase [84,85,88]. It is proposed by Davidson [87] that the reaction is initiated by a nucleophilic attack by the substrate amine nitrogen on the quinone carbon to give the carbinolamine intermediate (**3**); further evidence for this intermediate comes from resonance Raman spectroscopy [24,25,77]. The carbinolamine loses water to form the imino-quinone (**5**), which undergoes nucleophilic attack by an active-site base to give the carbanionic intermediate (**6**) concomitant with the reduction of TTQ. The X-ray structure of the enzyme indicates that the base is probably Asp-76 (Figure 10), and also shows that the C-6 carbonyl is the reactive carbonyl likely to undergo nucleophilic attack by the substrate [89]. This is close to a cation-binding site, and resonance Raman spectroscopy has indicated that the ammonium group of the substrate binds in this location adjacent to the C-6 carbonyl [90]. Hydrolysis of the carbanionic intermediate leads to release of the aldehyde product and formation of the aminoquinol (**11**). It is this final step that is very slow with benzylamines compared with the normal aliphatic substrates. My proposed reaction sequence in Figure 9 (adapted from Davidson's mechanism) involves an active-site base, not only in the key proton abstraction step (**5**–**6**) but also in the formation of the carbinolamine (**3**), the removal and subsequent addition of water, and the formation of the aldehyde product. Similarly, the oxidative half-reaction (see below) is also likely to involve an acid- or base-facilitated reaction during removal of the ammonia from the aminoquinol. It is likely, given the limitation of space in the active site, that a single residue is involved in most of these reactions.

Investigations using a number of inhibitors all support the mechanism described in Figure 9. Ammonia is a reversible competitive inhibitor, reacting to produce either an imino-quinone adduct [69,91] or a carbinolamine adduct [77,87]. The product depends on which enzyme is used, probably reflecting slight differences in the active site near the TTQ [77]. Various nucleophilic amines, such as phenylhydrazine, semicarbazide and hydroxylamine, are irreversible inhibitors, reacting with TTQ to form covalent adducts [65,69,92]. A likely mechanism for these reactions is the same as that with normal substrates, but there is no α-carbon for subsequent proton abstraction and so the reaction stops at the semiquinone stage (**5**) [92]. Cyclo-propylamine acts as an irreversible mechanism-based inhibitor, leading to covalent modification of TTQ and cross-linking of the α- and β-subunits [92]. This cross-linking indicates that the α-subunit must also play a role in providing the environment of the active site [92], and this is supported by the X-ray structure, which shows the TTQ located in a narrow channel at an interface between the subunits [93].

Oxidative half-reaction of methylamine dehydrogenase

The physiological electron acceptor (usually amicyanin) is a single-electron acceptor, leading to a semiquinone intermediate during oxidation of the aminoquinol that was produced in the reductive half-reaction (Figure 11). That a stable semiquinone intermediate is produced during the reaction has been known for some time [69,94], and this has been confirmed by resonance Raman spectroscopy, which also provided evidence for the formation of the hydroxy and carbinolamine adducts [77,78]. It was initially suggested that the first step in the oxidation of the

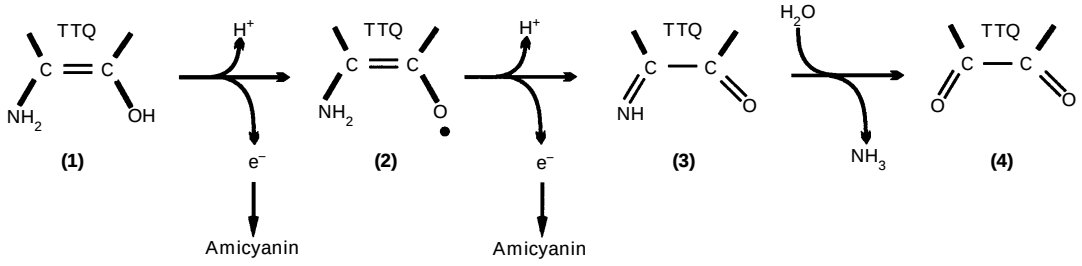


Figure 11 Oxidative half-reaction of methylamine dehydrogenase

The unpaired electron in (**2**) is delocalized in the indole ring of the TTQ. The hydrolysis of (**3**) to (**4**) is likely to involve an acid or base at the active site, which may be the same base(s) involved in the reductive half-reaction (Figure 9).

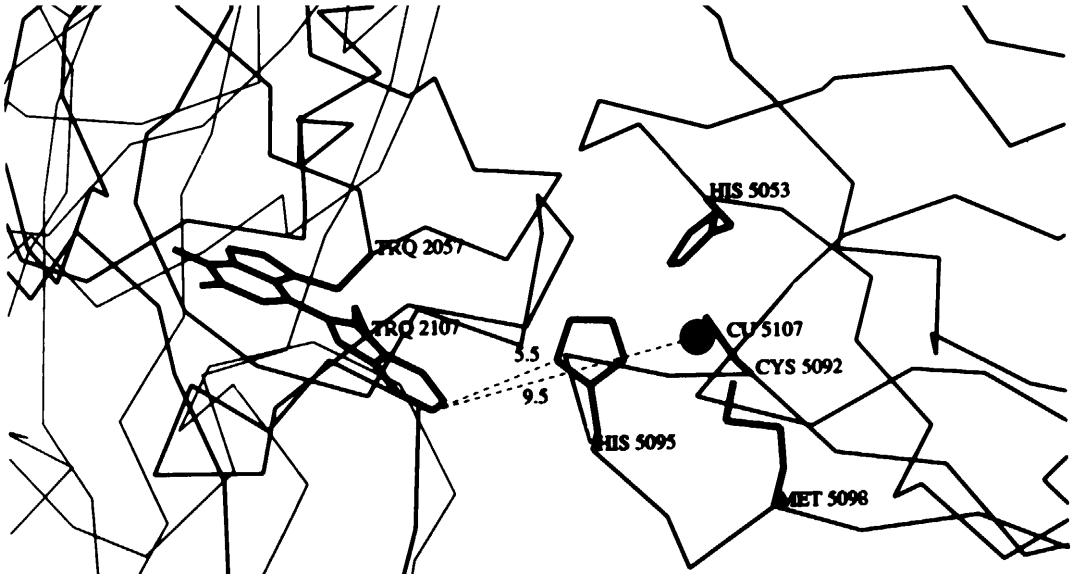


Figure 12 Interaction between methylamine dehydrogenase (left) and amicyanin (right)

The quinone part of the TTQ is on Trp-57 (TRQ 2057 in this Figure). The second tryptophan ring, Trp-108 (TRQ 2157 in this Figure) is nearer the electron acceptor (Cu 5107). The shortest distances between the TTQ and the copper atom and to the histidine (His-5095) are given in Å. Reproduced with permission from [138].

aminoquinol would involve the release of ammonia with production of the semiquinone, with the second electron transfer to amicyanin producing the oxidized quinone [72,95]. However, subsequent studies have shown that the nitrogen atom from the amine substrate is attached to the semiquinone, indicating that release of ammonia takes place after, or in concert with, the oxidation of the aminosemiquinone (Figure 11) [96,97,97a]. Spectroscopy of the aminosemiquinone revealed that it is deprotonated, and that the conformation of the tryptophans in relation to each other (dihedral angle of about 40°) is the same as in the crystal structure (Figure 10) [97]. These studies also showed that the delocalization of electrons into the ring system of the semiquinone is consistent with the route for departure of electrons from the indole ring that was proposed on the basis of the X-ray structure [98] and kinetic studies [99].

Three-dimensional structure of methylamine dehydrogenase and its complex with amicyanin

Methylamine dehydrogenase has an $\alpha_2\beta_2$ structure; the heavy α-subunits are approx. 40 kDa and the light β-subunits are approx. 13 kDa. Its three-dimensional structure has been determined using the enzymes from *Thiobacillus versutus* and *Paracoccus denitrificans* [76,93,100,101]. There is little interaction between the α-subunits of methylamine dehydrogenase, but extensive interactions exist between the α-and β-subunits, with the active site lying in a hydrophobic channel between them. The two indole rings of the TTQ are not co-planar, but lie at a dihedral angle of about 42° [101] (Figures 10 and 12). The larger α-subunit has a propeller-fold structure (seven-bladed), similar to that of the larger α-subunit of methanol dehydrogenase (eight-bladed) [22,30]. The fact that both of these quinoproteins have a propeller-fold structure is intriguing, because the related structures have no related catalytic function; in methanol dehydrogenase the prosthetic group (PQQ) is in the large subunit that has this propeller fold, but in methylamine dehydrogenase the TTQ is in the small subunit.

The complex formed between the dehydrogenase and amicyanin, solved by Mathews and his colleagues [98], has two molecules of amicyanin per $\alpha_2\beta_2$ tetramer of dehydrogenase; the amicyanin molecules have no contact with each other but are in contact with both subunits of the dehydrogenase. The greatest area of contact is between the amicyanin and the smaller β-subunit which contains the TTQ. Inhibition by high ionic strength had previously emphasized the importance of electrostatic interactions between the two proteins [102], but in the complex hydrophobic interactions predominate, perhaps reflecting the fact that the complex was crystallized in high-ionic-strength solutions. It is possible, however, that docking of the proteins is by electrostatic interactions and that formation of the specific electron transfer complex involves hydrophobic interactions, as has been suggested for the interaction of methanol dehydrogenase with its specific cytochrome *c* [48,49].

In the complex, the tryptophan that does not contain the *o*-quinone (Trp-108) lies near the surface, only 9.5 Å away from the amicyanin copper atom. His-95, one of the four copper ligands, is on the surface of the protein and about half-way between the copper and the TTQ, with the shortest distance between them being approx. 5.5 Å (Figure 12). It was therefore suggested that this histidine might mediate electron transfer between the redox centres, thus forming an electron transfer triad [98]. On the basis of stopped-flow kinetics, an electron transfer pathway has been suggested that involves a 3.6 Å jump through space from TTQ to the carbonyl of Pro-94 and passage through six covalent bonds to the copper (a total distance of 14 Å) [99]. An alternative pathway was suggested for electron transfer in a ternary complex with cytochrome *c*, which is moderately more efficient but depends critically on the presence of an intracomplex water molecule [45]. Measurements by polarized absorption spectroscopy of single crystals of these binary and ternary complexes have demonstrated that electron transfer between the redox centres does occur within the complexes [103], but some of the relatively low rates perhaps indicate that the orientation of the proteins is not necessarily optimal.

AMINE OXIDASES CONTAINING COPPER AND TPQ

Copper-containing amine oxidases catalyse the oxidative deamination of primary amines, the actual substrate (mono-, di- or poly-amine) depending on the source of the enzyme:

$$RCH_2NH_2 + O_2 + H_2O \rightarrow RCHO + H_2O_2 + NH_3$$

Although many amine oxidases with a great diversity of functions have been described, they are likely to be similar in overall structure and mechanism. They are important, for example, in processes as different as bacterial growth on amines, secondary metabolism in plants and the oxidation of histamine and neurotransmitters in animals. This diversity and its physiological and pharmacological significance has been described, together with extensive discussion of the history and mechanism of these enzymes, in comprehensive and authoritative reviews by Mondovi [104], McIntire and Hartmann [105], Knowles and Dooley [106] and Hartmann and Dooley [107].

Prosthetic group and copper site in amine oxidase

For nearly 60 years it has been known that amine oxidases have an organic, covalently bound, prosthetic group, and from about 1950 this has been assumed to be an unusual form of pyridoxal phosphate, as was also proposed for amine dehydrogenase. It was subsequently claimed to be a covalent form of PQQ, as also proposed for the amine dehydrogenases; this was a premature suggestion that proved to be incorrect, but which had the merit of directing attention to the problem of its true nature. This subsequently led Klinman and her colleagues [8,108,109] to identify the prosthetic group of bovine serum amine oxidase as the quinone of 2,4,5-trihydroxyphenylalanine (topa or 6-hydroxydopa); this is now known as TPQ (Figure 1). It should be noted that resonance Raman spectroscopy has demonstrated that there is substantial electron delocalization between the C-2 and C-4 oxygens and that only the C-5 oxygen has predominantly carbonyl character [110].

The review by Klinman and Mu [26] should be consulted for the authorized version of their exciting discovery of the structure of TPQ. The initial problem of obtaining sufficient active-site chromophoric peptide after proteolysis was overcome by first reacting the enzyme with [^{14}C]phenylhydrazine and then releasing the phenylhydrazone derivative by proteolysis using thermolysin in 2 M urea; the structure was then determined by MS and NMR of the pure peptide and synthetic compounds [8,111]. Resonance Raman spectra of the derivatized protein and the labelled peptide isolated from it were consistent with this structure [8,24,25,112], and this technique has been used to demonstrate TPQ in all the copper-containing amine oxidases examined [24,25,106]. A simple spectrophotometric assay has now been developed for identification of TPQ in amine oxidases which is based on the pH-dependence of the absorption spectra of TPQ *p*-nitrophenylhydrazone in the intact enzyme [108,113].

An extensive range of experiments and physical techniques has contributed to our knowledge of the structure of the copper site in amine oxidases, which has been summarized in an excellent recent review by Knowles and Dooley [106]. These techniques include EXAFS [3,114,115], ESR [115], electron nuclear double resonance [116], electron spin echo envelope modulation [117] and nuclear magnetic resonance dispersion [118,119] spectroscopy. From these investigations it was concluded that three histidine residues form ligands, together with two water ligands in a distorted square pyramidal configuration. Knowles and Dooley [106] pointed out that there was also the possibility that TPQ forms a weak axial co-ordination to the copper through one of its carbonyl oxygen atoms. During the turnover of the enzyme it is probable that an intermediate oxidation state is formed, with TPQ in the semiquinone form bonded to reduced Cu(I); there is, however, very little information available on the co-ordination of the copper centre in this oxidation state.

Reductive phase of the catalytic cycle of amine oxidase

Steady-state and stopped-flow kinetic studies have demonstrated an aminotransferase type of Ping-Pong mechanism [120]. The reaction with substrate leads to reduced enzyme and production of aldehyde; the enzyme is then oxidized by molecular oxygen with release of ammonia:

(i) $\text{Enz-CHO} + RCH_2NH_2 \rightarrow \text{Enz-}CH_2NH_2 + RCHO$

(ii) $\text{Enz-}CH_2NH_2 + O_2 + H_2O \rightarrow \text{Enz-CHO} + NH_3 + H_2O_2$

Perhaps not surprisingly, many features of the mechanism are similar to those described for the TTQ-containing amine dehydrogenases. Figure 13 gives a scheme for the reductive phase of the catalytic cycle, as deduced for the oxidation of benzylamines catalysed by bovine plasma amine oxidase. The reaction is initiated by a nucleophilic attack by the amine substrate, giving a carbinolamine intermediate (**3**) from which water is removed, leading to covalent attachment of substrate to TPQ at the C-5 carbonyl by way of a Schiff base (**5**), which can be trapped by reduction with $NaCNBH_3$ [121,122]. It was suggested that the low pK_a of the C-4 hydroxy group (~ 3) provides electrostatic stabilization of the initially formed Schiff base. Base-catalysed proton abstraction then gives a carbanion transition intermediate (**6**) [123–126]. The large primary isotope effect observed during benzylamine oxidation has led to the conclusion that significant quantum tunnelling occurs during this process. This is the first example of quantum mechanical tunnelling in an enzyme-catalysed proton abstraction mechanism [86,127], and it has subsequently been proposed for the TTQ-containing amine dehydrogenases. After formation of the carbanion (**6**) and transfer of electrons into the ring of TPQ, the pK_a of the C-4 hydroxy group undergoes a large increase, leading to proton transfer from the active-site base to the oxyanion at C-4 (**6**–**7**). This eliminates the electrostatic stabilization of the product Schiff-base imine complex (**7**), leading to its rapid hydrolysis and formation of the product aldehyde (**7**–**11**). It has been suggested that the presence of the C-4 hydroxy group increases the redox potential by 300 mV compared with a simple quinone such as dopa-quinone, thus increasing the driving force for the reaction [26,128]. The transient intermediate product Schiff base (**7**) was the most difficult intermediate to demonstrate, because of the rapid rate of its hydrolysis. It was eventually confirmed as its quinonoid tautomer by using benzylamines with electron-releasing substituents in rapid-scanning stopped-flow studies [129]. After release of the aldehyde product, the nitrogen from the substrate remains on the TPQ, which is thus now in the aminoquinol form [125,126,130]. Excellent comprehensive descriptions of the methods used for the synthesis and detection of the intermediates shown in Figure 13 have recently been published by Mure and Klinman [131] and Hartmann and Dooley [107].

In mechanisms of this sort, attention is usually directed towards the key catalytic step, which is proton abstraction from the α-carbon of the substrate. In addition to this step, I suggest that there are five other reactions that also require the involvement of an active-site base (or acid) (Figure 13). These include the transfer of the proton from the nitrogen to the oxygen (**2**–**3**), the removal of water from the resulting carbinolamine (**3**–**5**) and the

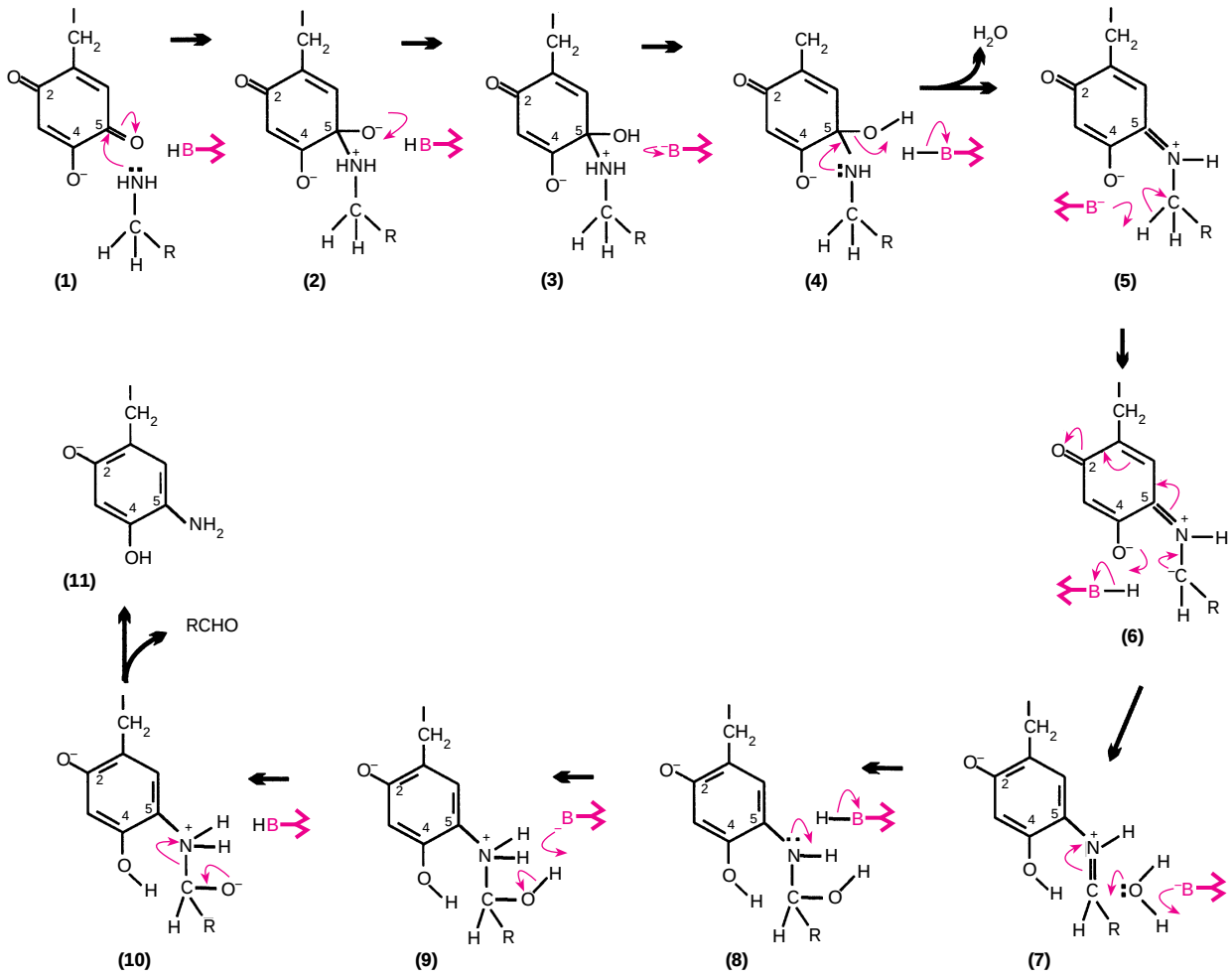


Figure 13 Reductive phase of the catalytic cycle of amine oxidase

Many of the intermediates are analogous to those in the mechanism for methylamine dehydrogenase shown in Figure 9. The key reaction is the proton abstraction by the active-site base (-B) from the α-carbon of the amine substrate. An active-site base (or possibly acid) is also likely to be involved in initial formation of the carbinolamine (**3**), removal of water from it to give the substrate Schiff base (**5**), addition of water to the product Schiff base (**7**) and production of aldehyde and the aminoquinol form of the prosthetic group (**11**).

later hydrolysis of the product Schiff base (**7**–**11**). It is possible that the same base (Asp-383) may be involved in all of these reactions. The same active-site base may also be involved in the removal of water from the aminoquinol product (**11**) during the oxidative half-reaction shown in Figure 14 (see below).

Oxidative phase of the catalytic cycle of amine oxidase

Oxidation of the aminoquinol form of TPQ, back to the quinone, involves the two-electron reduction of oxygen to hydrogen peroxide and the release of ammonia [120,132] (Figure 14). The inhibitory effects of azide or cyanide and their effects on the modification of the EPR spectrum provided evidence for the involvement of copper in this process [132,133], and established the occurrence of a free-radical intermediate form of enzyme containing the semiquinone form of TPQ and the reduced form of copper, Cu(I) [118]. Direct measurements of this intermediate led to the proposal that the reduced TPQ is oxidized by way of copper in two single-electron steps, the Cu(I)–semiquinone intermediate reacting with oxygen to form a transient superoxide intermediate [118,134–136]. It was initially proposed that the semiquinone form of TPQ is the iminosemiquinone [106,134], and this has been confirmed by resonance Raman spectroscopy [136]. Using the enzyme from *Escherichia coli*, and methylamine as a slow substrate, it was possible to show that the substrate amine group remains bound to the TPQ in the semiquinone state and that addition of cyanide leads to formation of a Cu(I)–cyanide complex with the nitrogen-containing semiquinone. Little is known about the actual site of oxygen reduction, and almost no information is available about the proton transfer steps that must be involved in oxygen reduction and peroxide release [106].

Active site of amine oxidase as seen in the X-ray structures of the *E. coli* and pea seedling enzymes

The first X-ray structure of a copper- and TPQ-containing amine oxidase has recently been obtained (at 2.0 Å) for the enzyme from *E. coli* by the group of Knowles and Phillips at Leeds [137]. Each subunit of the mushroom-shaped dimer consists of an extensive β-sandwich domain which contains the active site and provides the dimer interface, and three smaller α/β domains

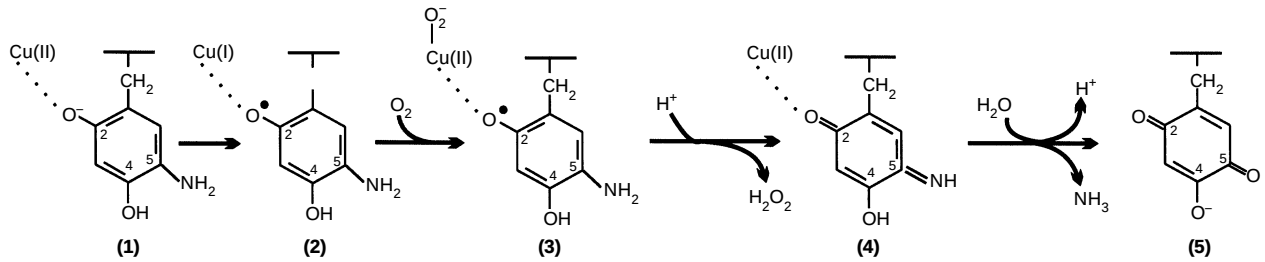


Figure 14 Oxidative phase of the catalytic cycle of amine oxidase

The copper is shown to be directly co-ordinated to the oxygen at position 2 of TPQ. If this distance is too great, a water molecule may come between the TPQ oxygen and the copper atom. A similar mechanism could be drawn, if structural observations suggest it, in which the copper is co-ordinated to the oxygen at position 4 of TPQ. The hydrolysis of intermediate (**4**) is likely to involve an active-site acid or base as discussed in the legends to Figures 9 and 13. This may or may not be the same base(s) involved in the reactions in Figure 13.

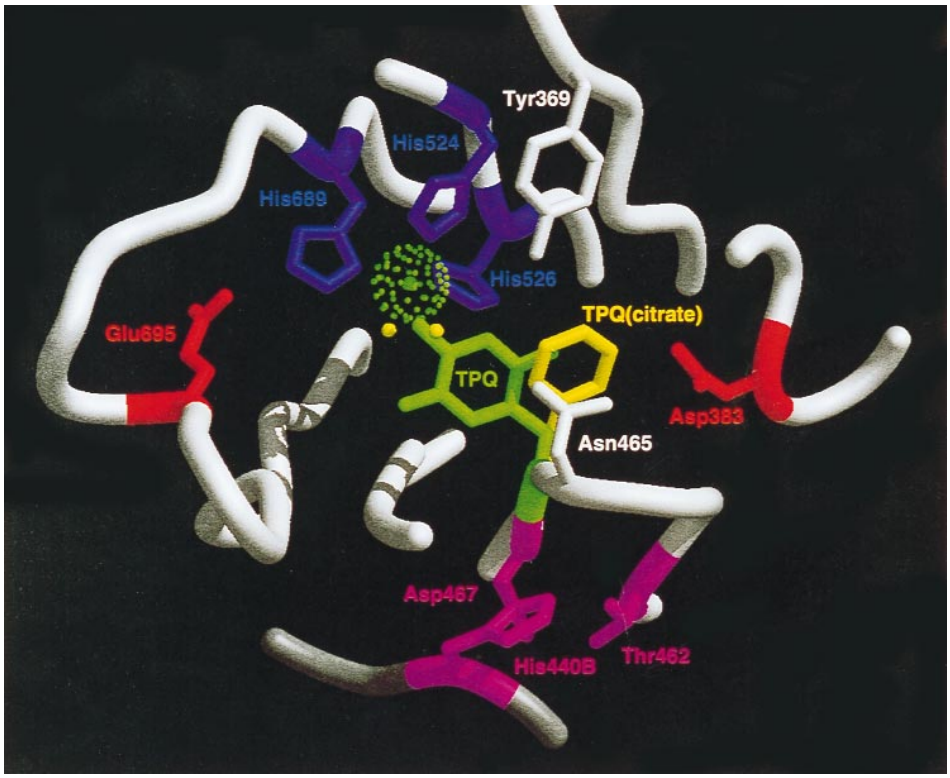


Figure 15 Active site of *E. coli* amine oxidase

Shown are the co-ordination of the copper, and the two conformations of the TPQ in the active sites of the two crystal types. In the enzyme crystallized from citrate, the TPQ (green) is co-ordinated to the copper. In the enzyme crystallized from ammonium salts, the precise location of the TPQ ring could not be completely determined and its general location is indicated by a yellow phenyl ring, close to the putative catalytic base (Asp-383, in red). In this form the TPQ is not a copper ligand and the copper co-ordination is completed by two water molecules, shown in yellow. Reproduced with permission from [137].

around the outside of the molecule. Two structures were solved, an active lower-resolution form and an inactive higher-resolution structure; these were essentially similar but with important differences in the active site. All of the identified residues of the active site are buried in the interior of the molecule between the sheets of a β-sandwich. The TPQ ring orientation is not clear in the active form, but it is orientated away from the copper and towards the putative active-site base Asp-383; it cannot be seen, however, which is the nearer out of the C-2 carbonyl and the C-5 carbonyl of TPQ, which is the carbonyl most likely to be involved in catalysis (Figure 15). In the inactive form, Asp-383 is within about 5.7 Å of O-2 of TPQ. Comparison with the pea seedling enzyme structure (see below) indicates that the possibility of rotation of the TPQ in the active site may be an important aspect of the catalytic function of these enzymes.

In the *E. coli* enzyme the copper atom lies about 12 Å below the molecular surface, co-ordinated to three histidine ligands (His-524, His-526 and His-689), their positions being very similar in the two crystal types. In the active enzyme, co-ordination of the copper is a distorted square pyramid, and is close to that proposed from spectroscopic studies described above. The apical ligand is water, and the three histidines plus a second water

molecule form the distorted base of the pyramid (Figure 15); TPQ was not within co-ordination distance in this active form of the enzyme. In the inactive form there was no water in the co-ordination sphere of the copper. The co-ordination geometry was described as distorted tetrahedral, with His-524 in an 'axial-like' position and the other three ligands 'equatorial-like'; these ligands are His-526 and His-689 plus TPQ co-ordinated via the oxygen at the 4-position. There was no electron density to suggest the presence of a second axial-like ligand, a small empty pocket being present at this location.

The first structure of a eukaryotic (pea seedling) amine oxidase has now been solved at 2.2 Å [139] and shown to be very similar to that of the *E. coli* enzyme. The TPQ aromatic group is located approx. 6 Å away from the Cu atom, its location being different from that in both the active and inactive forms of the *E. coli* enzyme. It has been suggested [139] that the combination of evidence from the two structures indicates that the TPQ side chain is sufficiently flexible to permit the aromatic group to rotate about the Cβ–Cγ bond, and to move between bonding and non-bonding positions with respect to the Cu atom. Some conformational flexibility is also required at the surface of the molecule to allow the substrates access to the active site, which is inaccessible to solvent, as expected for an enzyme that uses radical chemistry. The potential for rotation of the TPQ is also likely to be relevant to the mechanism for production of the modified tyrosine which involves the copper atom at the active site [139].

LYSYL OXIDASE: A SPECIAL AMINE OXIDASE CONTAINING COPPER AND LTQ

Lysyl oxidase plays a major role in the development and repair of connective tissues. It catalyses the oxidation of lysyl residues in collagen and elastin to form the peptidyl α-aminoadipic-δ-semialdehyde:

$$\text{Protein-}(CH_2)_3\text{-}CH_2\text{-}NH_2 + O_2 \rightarrow \text{Protein-}(CH_2)_3\text{-}CHO + H_2O_2 + NH_3$$

Aldehyde residues produced by this reaction spontaneously condense with nearby aldehydes or ϵ-amino groups, giving inter- or intra-chain covalent cross-linkages, thus leading to the insoluble fibres in the extracellular matrix [140]. Earlier work indicated that the enzyme contains copper and that catalysis involves a carbonyl group; most subsequent work on its mechanism has indicated that it is very similar to other copper-containing amine oxidases [140]. The discovery of TTQ as the prosthetic group of amine oxidases drew attention to the possibility that lysyl oxidase also contained TTQ, although those working in the field were sensibly wary of warmly embracing this idea (see [141]). The recent publication of the structure of the prosthetic group of lysyl oxidase by Klinman, Dooley, Kagan and their colleagues [8a] has justified this caution. The structure is derived from the cross-linking of the ϵ-amino group of a peptidyl lysine with the modified side chain of a tyrosyl residue, and has been designated LTQ (Figure 1). It appears to be the only example of a mammalian cofactor formed from the cross-linking of two amino acid side chains. It has been pointed out [8a] that alternative TPQ analogues might occur in which other amino acids substitute for the lysyl residue in LTQ. There is some evidence that this does, indeed, occur in the prosthetic group of one of the amine oxidases of the fungus *Aspergillus niger*; in the isolated prosthetic group of this enzyme the modified tyrosine residue is esterified to the γ-carboxyl of glutamate [142].

CONCLUDING REMARKS

This review summarizes the results of a great range of different approaches that have been applied to the study of the mechanisms of the four types of quinoprotein, and the conclusions from this work have been satisfyingly supported by the three-dimensional structures elucidated by X-ray crystallography. As expected, the quinone structures in the prosthetic groups play important roles in all of the mechanisms. A second common feature is the presence of a catalytic base (aspartate) in the active sites which initiates the reactions by abstracting a proton from the substrate; it is also apparent that this same base is likely to be involved in multiple reactions in all of the mechanisms. Another common feature of these enzymes is that the first part of the reaction produces a reduced prosthetic group, this part of the mechanism being fairly well understood; this is followed by an oxidative phase involving electron transfer reactions which are far less well understood. In the case of the dehydrogenases, electrons must pass one at a time from the prosthetic group to redox centres in a second recipient protein (or protein domain), whereas in the amine oxidases electrons must be transferred to molecular oxygen by way of a redox-active copper ion in the enzyme itself. It is this area that is most likely to occupy future investigators, together with attempts to understand further the details of the enzyme mechanisms; this must include determining the location of the substrates and activators, and understanding their interactions in the active sites. This review has been written at the start of a new phase in the study of these fascinating proteins in which a wide range of physical techniques will continue to be focused upon the problem, coupled with extensive studies using the techniques of site-directed mutagenesis and X-ray crystallography.

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