Introduction
This is a brief review of those dehydrogenases that have as their prosthetic group pyrroloquinoline quinone (PQQ), the best-known being those that catalyse the oxidation of carbohydrates and alcohols. For comprehensive recent reviews of their structure and function see [1,2]; for biochemical, physiological and genetic aspects of PQQ and quinoproteins see [4,5]; for reviews discussing mechanisms of all types of quinoprotein see [5,6], and see Davidson [7] for a good overall survey of all aspects of quinoproteins.

Although they all catalyse reactions in the bacterial periplasm, there is considerable diversity in the nature of these dehydrogenases. They may also contain haem as a second prosthetic group (the quinohaemoproteins); they may be monomeric or multimeric; freely soluble in the periplasm or bound to membranes; some are firmly associated with other redox components while others are not; their electron acceptors may be cytochromes, blue copper proteins or membrane ubiquinone; the PQQ may be tightly bound (non-covalently) or easily dissociated.

When it has been investigated, it has been shown that all these dehydrogenases depend on a divalent metal ion for their activity which, in methanol dehydrogenase, is Ca\(^2+\) bonded to PQQ.
in the active site [8,9]. The quinoproteins may be assayed by using their physiological electron acceptors or by using artificial dyes such as phenazine ethosulphate or Wurster’s Blue which are assumed to interact directly with the PQQ or to accept electrons from the surface of the enzyme near the PQQ. In addition to using these electron acceptors, the quinohaemoproteins may also be assayed with ferricyanide, which accepts electrons from the higher-potential haem groups. For references to assay systems see [5,6,10,11].

The diversity of PQQ-containing quinoproteins and their associated electron transport chains

The quinoprotein dehydrogenases function to provide ATP by coupling periplasmic oxidation reactions to the reduction of molecular oxygen by way of a diversity of electron transport chains [4,12,13] (Figure 1). The questions that are important in understanding these proteins are essentially those of interest in the study of all electron transfer systems, including the following: the mechanism of reduction by substrate of the prosthetic group; interaction of the dehydrogenase with its electron acceptor, which is often a second protein; electron transfer within the dehydrogenase; electron transfer pathways between redox proteins.

The best characterized of these dehydrogenases is the periplasmic soluble alcohol dehydrogenase in methanol oxidizing bacteria, being the only PQQ-containing quinoprotein for which the X-ray structure has been published [8,9]; its electron acceptor is a specific cytochrome, cytochrome c₁. A similar ethanol dehydrogenase, which also uses a specific cytochrome c electron acceptor, has been described in typical heterotrophic bacteria such as Pseudomonas [4,14]. A second type of periplasmic soluble alcohol dehydrogenase (in Comamonas testosteroni) is a quinohaemoprotein, containing a haem molecule in addition to PQQ; it is assumed to use a specific cytochrome c or blue copper protein as its electron acceptor [15,16]. A third type of alcohol dehydrogenase is the membrane-associated quinohaemoprotein alcohol dehydrogenase from acetate oxidizing bacteria (Acetobacter and Gluconobacter); its electron acceptor is ubiquinone in the membrane [17–20]. The other main type of PQQ-containing quinoprotein is the membrane-bound glucose dehydrogenase, the electron acceptor for which is ubiquinone; it occurs in a wide range of bacteria including enteric bacteria, Acinetobacter and acetic acid bacteria [4,13,21].

The structure of the PQQ-containing quinoprotein dehydrogenases

The only structure determined is that of methanol dehydrogenase [1,2,8,9]. The basic structure of the α subunit is a ‘propeller fold’ superbarrel made up of eight β sheet ‘propeller blades’ (W motifs) which are held together by novel tryptophan docking motifs (Figure 2A). There is also a small β subunit which wraps around the α subunit. The PQQ is in the centre of the α subunit, coordinated to a Ca²⁺ ion (Figure 2B) and is maintained in position by a stacked tryptophan and a novel eight-membered ring structure made up of a disulphide bridge between adjacent cysteine residues. All other PQQ-containing quinoproteins have regions of sequence corresponding to the methanol dehydrogenase super-
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Figure 2
Methanol dehydrogenase

(A) Single αβ unit showing the radial symmetry of the superbarrel. (B) Equatorial interactions of PQQ and the coordination of Ca²⁺ in the active site.

barrel. The quinohaemoprotein alcohol dehydrogenases have a long C-terminal domain containing the haem, and the glucose dehydrogenase has an N-terminal membrane anchor domain. It has been possible to use the sequences of the alcohol dehydrogenase [20] and glucose dehydrogenase [21], together with the coordinates of the methanol dehydrogenase, to produce reliable model structures of the 'superbarrel regions' of these two enzymes, the model structure of the glucose dehydrogenase being used as a basis for site-directed mutagenesis [21,22]. A key feature enabling this to be done is the high level of conservation of the tryptophan docking motifs which stabilize the basic structure of the superbarrel.

The mechanism of reduction of PQQ in the active site
This has been studied mainly in methanol dehydrogenase [5,6,10,11]. The orthoquinone form of PQQ is reduced by methanol to the quinol which is then oxidized by two sequential single-electron transfers to cytochrome c₅ by way of the free radical semiquinone. Figure 2(B) shows the active site of the enzyme with the Ca²⁺ bonded to the oxygen of the C₅ carbonyl of PQQ. It is suggested that the base (Asp-303) abstracts a proton from methanol and that the Ca²⁺ ion facilitates attack by the resulting oxy-anion on the electrophilic C5, to give the hemiketal from which the methyl proton is abstracted; this is facilitated by ionization of the C4 carbonyl oxygen which is made possible by the pyrrole nitrogen atom. An alternative mechanism is possible in which the initial proton abstraction is the same, but the electrophilic C5 is involved directly in removal of the methyl hydrogen as hydride. In a study of the role of the metal ion in the active site, we have used enzyme prepared from a processing mutant to produce a unique enzyme in which the Ca²⁺ is replaced with Ba²⁺; this has a much reduced affinity for methanol but its activation energy is halved [23,24]. When the proposed catalytic base, Asp-303, was changed to glutamate by site-directed mutagenesis, the enzyme remained highly active but it had a very much lower affinity for the substrate. The reaction mechanism of the other dehydrogenases is likely to be similar, and site-directed mutagenesis of the equivalent base in the glucose dehydrogenase of Escherichia coli showed that activity was lost when it was converted into asparagine, but that active enzyme with low affinity for glucose resulted when this residue was
Electron transfer reactions involving PQQ-containing quinoproteins

Methanol dehydrogenase and cytochrome $c_1$, 'dock' together initially by electrostatic interactions between a small number of lysyl residues on the dehydrogenase and carboxylates on the cytochrome [12]. Surprisingly this is not inhibited by 50 μM EDTA which, although not inhibiting the dehydrogenase reaction, inhibits the overall electron transfer process between the proteins. We have suggested, therefore, that EDTA inhibits by binding to lysyl residues, 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 [25,26]. The structure of cytochrome $c_1$ [27] confirms that the region around the haem edge, where electron transfer is likely to occur, is similar to that in other $c$-type cytochromes in being hydrophobic and therefore able to be involved in docking with the dehydrogenase hydrophobic funnel region.

Electron transfer from the quinol form of PQQ to the cytochrome electron acceptor occurs in two single-electron transfer steps — the semiquinone form of PQQ being produced after the first of these transfers. An obvious candidate as an intermediary in this process is the novel disulphide bridge between adjacent cysteines in the active site. However, this has been ruled out by our demonstration that reduction of the bridge followed by carboxymethylation yields active enzyme [28]. Although this very rare structure is present in all the alcohol dehydrogenases it is absent from glucose dehydrogenase in which electrons are transferred to membrane ubiquinone 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 the stabilization, or protection from solvent at the entrance to the active site, of the free-radical PQQ semiquinone in methanol dehydrogenase.

After the cytochrome $c_1$, leaves the dehydrogenase it transfers electrons to the typical class I $c$-type cytochrome (cytochrome $c_0$). The overall structure of this cytochrome is similar to others in its class but it has some unique features [29] which will make a study of its interaction with cytochrome $c_1$, of great interest.

Although its structure has not yet been determined, the type II soluble quinohaemoprotein alcohol dehydrogenase (from Comamonas testosteroni) is likely to provide an excellent system for the study of intra-protein electron transport. The haem iron in this quinohaemoprotein is similar to that in other low spin cytochromes $c$ in being coordinated by histidine and methionine [15]. The apo-enzyme requires the addition of PQQ for activity and this has been used to show that binding of PQQ induces a conformational change in the protein, a reorientation of the methionine ligand of haem C, an increase of electron density on one of the pyrrole rings and an increase in midpoint redox potential of the haem [16]. All of the available evidence is consistent with the obvious interpretation, that electrons pass, directly or indirectly, from the reduced PQQ to the haem (midpoint redox potential = 140 mV), and thence to an external electron acceptor which is likely to be a high-potential $c$-type cytochrome or a blue copper protein.

The type III alcohol dehydrogenase of acetic acid bacteria is a quinohaemoprotein–cytochrome $c$ complex, having three subunits tightly bound to the periplasmic membrane. The electron acceptor is membrane ubiquinone, so we have the unusual situation in which a $c$-type cytochrome precedes ubiquinone in an electron transport chain. Modelling the large catalytic domain of subunit I shows that it is similar to methanol dehydrogenase [20], but it has a C-terminal extension bearing a single haem C; subunit II is a cytochrome with three haems C; subunit III is small and not present in all enzymes of this type. Electron transfer from the reduced PQQ to membrane ubiquinone is via the haem on the same quinohaemoprotein subunit and two or three of the haems in the cytochrome subunit II [17], which is embedded in the membrane. The role of subunit III seems to be to help the dehydrogenase subunit I couple with the cytochrome $c$ (subunit II), thereby maintaining the correct conformation for electron transport in the complex on the periplasmic surface of the membrane [17–19]. An important question remaining is how the ubiquinone in the membrane reacts with subunit II to accept electrons from its haem. Clearly part of the protein must be embedded in the membrane for this to occur...
but subunit II does not appear to have typical hydrophobic transmembrane helices.

In the membrane glucose dehydrogenase, electrons must pass from the reduced PQQ, in the catalytic site buried in the periplasmic domain, to ubiquinone which presumably has a binding site in the membrane domain; the membrane domain is made up of four to five transmembrane α helices [30].

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