Substrate binding in Quinoprotein Ethanol Dehydrogenase studied by Multi-Frequency EPR & ENDOR

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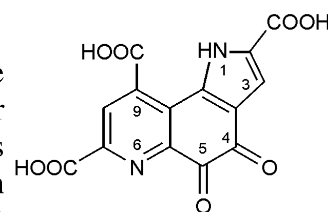
Pyrroloquinoline quinone (2,7,9-tricarboxypyrroloquinoline quinone, PQQ; Scheme 1) is one of several quinone cofactors utilized in a class of alcohol and glucose Dehydrogenases, known as quinoproteins, which are distinct from the flavin- and nicotinamide-dependent oxidoreductases (1,2).

The quinoprotein Methanol Dehydrogenase (MDH) is among the best-characterised PQQ-dependent enzymes (3). The PQQ cofactor bound to a Ca^{2+} ion is buried in the interior of the protein and is sandwiched between the indole ring of a tryptophan residue and an eight-membered disulfide ring structure formed from adjacent cysteine residues that is unique to this class of enzymes.



Scheme 1. Structure of PQQ.

In order to elucidate reaction mechanisms, emphasis is often placed on obtaining protein structures in the presence of substrate by x-ray crystallography. For Glucose Dehydrogenase a structure has been reported with a molecule of glucose bound in the active site (4), whereas in alcohol Dehydrogenases obtaining structures with substrate has proven more difficult (3). In the Quinohemoprotein QEDH from *Pseudomonas putida*, acetone (the oxidation product of isopropanol) was found in the active site (5). In QEDH from *P. aeruginosa*, several alcohols were modeled into the empty active site (6).

In this contribution we have used EPR and ENDOR at 9.7 GHz (X-Band) and 94 GHz (W-Band) to study substrate binding in QEDH, taking advantage of the fact that the enzyme is isolated with a substantial proportion of the PQQ cofactor in the semiquinone form. High-field EPR has an advantage over conventional 9 GHz EPR (X-Band) in that the anisotropy of the g-tensor of organic radicals, such as quinones, flavins and nitroxides is often resolved. Thus, it is possible to detect changes in the radical environment, such as polarity or hydrogen bonding, by observing shifts in the g-anisotropy in the presence and absence of substrate. We have also used pulsed ENDOR and HYSCORE at X-Band to determine hyperfine couplings of the PQQ cofactor, again in the presence and absence of ethanol. Hyperfine couplings from the ethanol substrate could also be detected and assigned to the different groups of protons by selective deuteration, allowing the distance between the substrate and cofactor to be estimated.

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