An Active Site Model for Calcium(II)-containing Quinoproteins

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The Ca^{2+} complexes of **PQQ**-2,9-dimethyl ester and its iminoquinone derivative have been synthesised and the reactivity towards alcohols examined; the oxidising ability of the quinone is significantly enhanced by binding Ca^{2+} and NH_3, both of which are essential for quinoprotein methanol dehydrogenase activation.

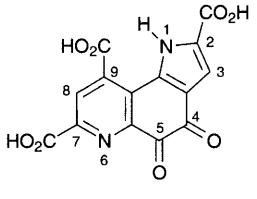
PQQ (4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid) is a novel cofactor of several NAD(P)- or flavin-independent dehydrogenases involved in the oxidation of alcohols and aldose sugars in bacteria.[1] The structure of **PQQ** has attracted much attention because of its potential ability as a metal ligand,[2] although the interaction of **PQQ** and metal ions in living systems has not been identified until recently. Recent X-ray crystallographic studies of quinoprotein methanol dehydrogenase (MEDH) have shown that the cofactor **PQQ** directly coordinates to Ca^{2+} through the C-5 carbonyl oxygen, N-6 pyridine nitrogen and C-7 carboxylate group at the enzyme active centre.[3] A similar interaction of Ca^{2+} and **PQQ** cofactor has also been suggested with ethanol- and glucose-dehydrogenase.[4,5] Davidson and coworkers have reported the important role of Ca^{2+} in the structural stabilisation of the enzymes,[6] but nothing is known about the catalytic role of Ca^{2+} for the enzymatic redox reactions. Here we report the synthesis and reactivity of the first Ca^{2+} complexes of **PQQ** and their iminoquinone derivatives to try to shed light on the catalytic roles of Ca^{2+} and NH_3, both of which are known activators of quinoprotein methanol dehydrogenase.

We used **PQQ**-2,9-dimethyl ester **1** which retains the functional groups (C-5 quinone carbonyl, N-6 pyridine nitrogen and C-7 carboxyl group) for Ca^{2+} binding. Hydrolysis of **PQQTME** (the trimethyl ester of **PQQ**) with CF_3CO_2H/H_2O at 60 °C for 12 h gave the expected 2,9-dimethyl ester in 67% yield (Scheme 1).† Addition of $Ca(NO_3)_2$ (10 equiv. in MeCN) to an MeCN solution of **1** (5.6×10^{-3} mol dm^{-3}) quantitatively gave the Ca^{2+} complex **2** as a red powder.†

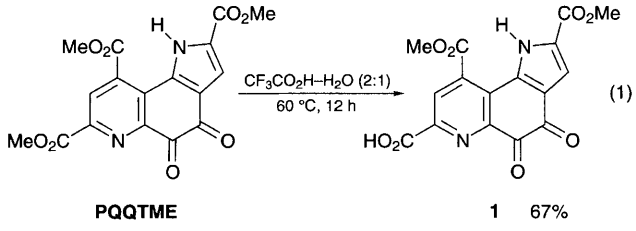
However, the same reaction using **PQQTME** did not give the expected product clearly indicating that the carboxyl group at the 7-position plays an essential role in the Ca^{2+} binding. In the IR spectrum, there is a strong absorption at 1628 cm^{-1} showing that the carboxyl group is in the carboxylate form to bind Ca^{2+}. The strong IR absorption at 1392 cm^{-1} together with the small ones at 824 and 738 cm^{-1} indicate that the nitrate ion acts as a bidentate ligand.[7] The IR absorption of the quinone carbonyl group of **1** (1690 cm^{-1}) shifts slightly (1684 cm^{-1} in **2**) by the complex formation, and the UV–VIS absorption at around 440 nm due to the n-π* transition of the *o*-quinone function of **1** also shifts by *ca.* 50 nm with the Ca^{2+} complex **2** (490 nm). Such spectral changes also suggest the interaction between Ca^{2+} and the quinone carbonyl group. The existence of two water molecules in the complex was suggested by elemental analysis; they may be ligating rather than lattice water molecules, since no free water molecule peaks were detected in the TG analysis below 300 °C. All these results support the structure of Ca^{2+} complex **2**. The similar O-rich coordination environment (5 O and 1 N) for Ca^{2+} has been reported in the MEDH active centre.[3] Crystal structures of other metal ion complexes of **PQQ** or its analogues so far reported all suggest that the region around the pyridine nitrogen is the best place for any metal ion.[2a,c,d,g,h]

It has been reported that MEDH requires NH_3 or a primary amine as an activator of the enzyme.[8] In order to obtain information about the catalytic role of NH_3, we prepared an iminoquinone derivative of the calcium complex. Treatment of compound **2** (3.5 mg, 7.6 μmol) with NH_3 in MeCN containing 1% DMSO (3.5 ml) gave a dark green solid **3** (86%).† Transformation of **PQQTME** to the corresponding C-5 iminoquinone derivative caused large upfield shifts of H-3 and H-8 in the ^1H NMR spectrum (**PQQTME**: H-3, δ = 7.28; H-8, 8.61, C-5 iminoquinone: H-3, 7.08; H-8, 7.94).[9] Similar chemical shifts were observed in the case of compounds **2** and **3** (**2**: H-3, δ = 7.21; H-8, 8.41, **3**. H-3, 6.97; H-8, 7.87). The appearance of the IR absorption band at 1660 cm^{-1} corresponding to the C=N function also provides evidence for iminoquinone formation. Existence of the bidentate NO_3^- ligand was also shown by a strong IR absorption band at 1386 cm^{-1} and the weaker ones at 810 and 764 cm^{-1}. Instability of compound **3** towards hydrolysis, however, precludes the identification of other coordinated molecules such as water and/or ammonia.

Neither **PQQTME** or compound **1** is reactive towards benzyl alcohol. On the other hand, the Ca^{2+} complex **2** does oxidise benzyl alcohol to benzaldehyde as shown in Table 1. The oxidising ability of the iminoquinone Ca^{2+} complex **3** for benzyl alcohol is drastically enhanced as compared to that of the others (oxidation yield: 80%). Although the details of the alcohol oxidation mechanism is not yet clear, there is a correlation between the oxidation ability and the equilibrium constant (K_{add}) for the hemiacetal formation with methanol.[10] This may suggest that the oxidation of benzyl alcohol to benzaldehyde

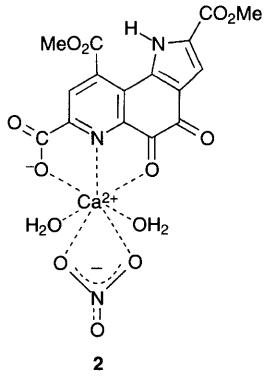


PQQ



PQQTME **1** 67%

Scheme 1



2

Table 1 Equilibrium constants (K_{add}) for hemiacetal formation with methanol and the oxidation yields of benzyl alcohol

Quinone	K_{add}/dm^3 mol^{-1} [a]	Yield of PhCHO (%)[b]
PQQTME	0.63	0
1	0.75	0
2	1.55	11
3	39.7	80

[a] Determined by UV–VIS titration in MeCN according to the reported procedure.[9] [b] Quinone (1×10^{-3} mol dm^{-3}), $PhCH_2OH$ (0.1 mol dm^{-3}), in MeCN containing 15% DMSO at 25 °C for 24 h under Ar. The yields (±5%) were determined by GLC based on the quinone.

proceeds *via* a polar addition–elimination mechanism as in the amine oxidation by **PQQTME**.[9]

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Footnote

† *Physical and spectroscopic data* for **1**: mp 219–221 °C; ^{1}H NMR (DMSO-[$^2H_6$]) δ 3.89 (3 H, s, CO_2Me), 4.05 (3 H, s, CO_2Me), 7.28 (1 H, s, H-3), 8.56 (1 H, s, H-8) and 12.52 (1 H, brs, H-1); ^{13}C NMR (DMSO-[$^2H_6$]) δ 52.31, 54.15 (CO_2CH_3 × 2), 113.91, 124.80, 126.28, 126.36, 128.45, 133.72, 134.06, 146.95, 148.85 (aromatic carbon × 9, 159.83, 164.73, 166.70 (CO_2H and CO_2CH_3 × 2), 173.31 (d, ^3J = 1.5 Hz, C-4) and 177.25 (s, C-5); ν (KBr)/cm^{-1} 3236 (OH), 1752 (CO_2H), 1718 (CO_2Me) and 1690 (quinone C=O); λ_{max} (MeCN)/nm 258 (ε 23700 dm^3 mol^{-1} cm^{-1}), 357 (12900) and 440 (sh) (1650); *m/z* (EI) 358 (M^+). The position of the carboxyl group in **1** was confirmed by comparing the IR and ^{1}H NMR spectral data and the physical data such as pK_as of the carboxyl group and the pyrrole proton with those of the 2,7-dimethyl ester derivative of **PQQ**. For **2**: mp > 300 °C; ^{1}H NMR δ (DMSO-[$^2H_6$]) 3.83 (3 H, s, CO_2Me), 4.02 (3 H, s, CO_2Me), 7.21 (1 H, s, H-3), 8.41 (1 H, s, H-8) and 12.75 (1 H, brs, H-1); ν (KBr)/cm^{-1} 1722 (ester carbonyl), 1684 (quinone carbonyl), 1628 (carboxylate), 1392, 824 and 738 (bidentate NO_3^-); λ_{max} (MeCN containing 0.6% DMSO)/nm 257 (ε 25300 dm^3 mol^{-1} cm^{-1}), 360 (12800), 490 (sh) and (900); *m/z* (FAB, positive) 398 ($M^+ + 1 - NO_3^-$). For **3**: mp > 300 °C; ^{1}H NMR δ (DMSO-[$^2H_6$]) 3.68 (3 H, s, CO_2Me), 3.92 (3 H, s, CO_2Me), 6.97 (1 H, s, H-3) and 7.87 (1 H, s, H-8); ν (KBr)/cm^{-1} 1714 (CO_2CH_3), 1660 (C=N), 1622 (CO_2^-), 1386, 810 and 764 (bidentate NO_3^-); λ_{max} (MeCN)/nm 290 (ε 18600 dm^3 mol^{-1} cm^{-1}) and 361 (12400); *m/z* (FAB, positive) 397 ($M^+ + 1 - NO_3^-$).

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