Catalysis by calcium ion of the reoxidation of reduced PQQ by molecular oxygen

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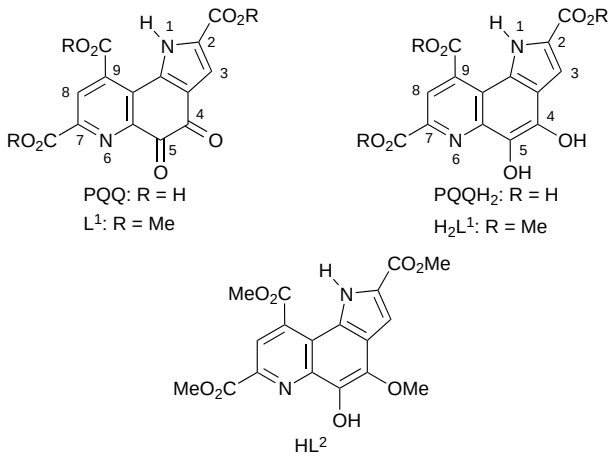
Reoxidation of reduced PQQ to the quinone by molecular oxygen is enhanced drastically by Ca^{2+}, the interaction of which has been recently demonstrated to exist in the enzyme active site by the X-ray crystallographic analysis of quinoprotein methanol dehydrogenase.

PQQ (pyrroloquinolinequinone) is a novel coenzyme that was first isolated and identified from bacterial methanol dehydrogenases (MDH, E.C. 1.1.99.8) in 1979.[1] This coenzyme is the redox centre of MDH that catalyses the oxidation of methanol to formaldehyde and donates two electrons to a c-type cytochrome in biological systems.[2] Recently, the crystal structure of MDH from methylotrophic bacteria has been determined by two independent research groups to provide full particulars of the enzyme active centre. According to the reported X-ray structure, there is one calcium ion strongly bound to PQQ through its C-5 quinone carbonyl oxygen, N-6 pyridine nitrogen, and C-7 carboxylate group in the enzyme active site.[3,4]† In this context, we have recently demonstrated that the oxidation of methanol to formaldehyde by coenzyme PQQ is promoted significantly by Ca^{2+}, providing valuable insight into the role of Ca^{2+} in the enzymatic alcohol-oxidation mechanism.[5] On the other hand, however, very little is known about the catalytic role of Ca^{2+} in the reoxidation process of reduced PQQ. Here, we report that Ca^{2+} also enhances the oxidation of reduced PQQ to the quinone by molecular oxygen, providing further information on the catalytic function of Ca^{2+} in the enzymatic redox reactions.

The trimethyl ester of reduced PQQ in the quinol form (H_2L^1, 2.5×10^{-5} mol dm^{-3})[6] is oxidised by molecular oxygen very slowly in anhydrous acetonitrile (MeCN) as shown in Fig. 1 (line *a* in the inset). Addition of Ca^{2+} (8.2×10^{-3} mol dm^{-3}) into the O_2-saturated MeCN solution of H_2L^1 results in significant acceleration of the oxidation rate (line *b* in the inset of Fig. 1). The absorbance at λ_{max} = 326 nm due to H_2L^1 decreases accompanied by the increase in absorbance due to the quinone L^1 (Fig. 1). Quantitative formation of H_2O_2 in the final reaction mixture has been confirmed by iodometric titration using NaI. Rates of the reaction of H_2L^1 and O_2 in the presence of various concentrations of Ca^{2+} in MeCN at 298 K were determined by monitoring the decrease in absorbance at λ_{max} = 326 nm due to H_2L^1. The rates obeyed pseudo-first-order kinetics in the presence of a large excess of O_2 (1.3×10^{-2} mol dm^{-3}) and the observed pseudo-first-order rate constant (k_{obs}) increases with an increase in [Ca^{2+}] to exhibit first-order dependence on [Ca^{2+}] at low concentrations, changing to second-order dependence at high concentrations, as shown in Fig. 2.



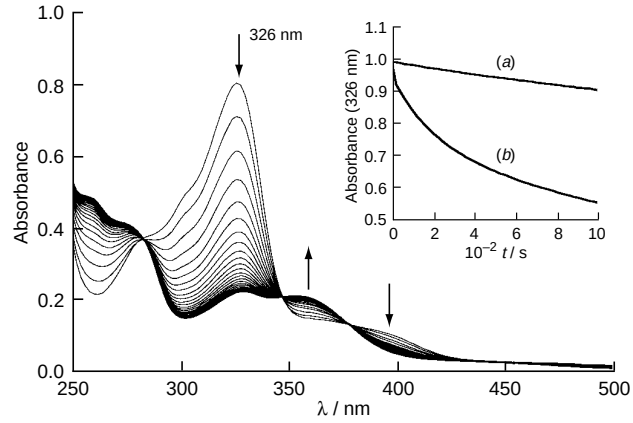


Fig. 1 Spectral changes observed in the oxidation of H_2L^1 (2.5×10^{-5} mol dm^{-3}) by O_2 (1.3×10^{-2} mol dm^{-3}) in the presence of $Ca(ClO_4)_2$ (8.2×10^{-3} mol dm^{-3}) in O_2-saturated MeCN containing 0.8% Me_2SO at 298 K. Inset: time course of the reaction followed at λ = 326 nm under an O_2 atmosphere (*a*) in the absence and (*b*) in the presence of $Ca(ClO_4)_2$ (1.0×10^{-3} mol dm^{-3}) at 298 K.

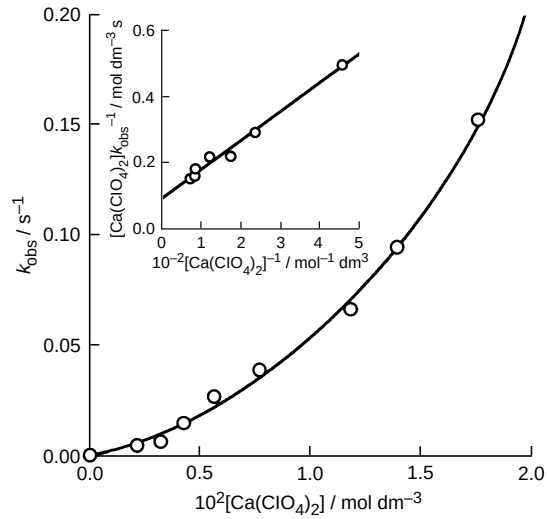
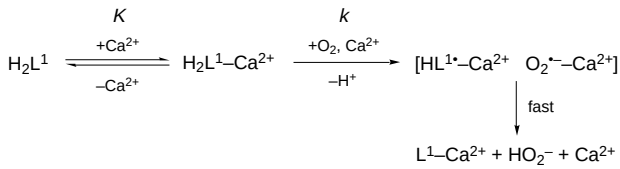


Fig. 2 Plot of k_{obs} *vs.* [$Ca(ClO_4)_2$] for the oxidation of H_2L^1 (2.5×10^{-5} mol dm^{-3}) by O_2 (1.3×10^{-2} mol dm^{-3}) in the presence of $Ca(ClO_4)_2$ in O_2-saturated MeCN containing 0.8% Me_2SO at 298 K. Inset: plot of [Ca^{2+}]k_{obs}^{-1} *vs.* [Ca^{2+}]$^{-1}$ for the reaction.

Addition of $Ca(ClO_4)_2$ to a deaerated MeCN solution of H_2L^1 results in a decrease in absorbance at λ_{max} = 326 nm due to H_2L^1 accompanied by an increase in absorbance at λ_{max} = 350 nm due to the Ca^{2+} complex of a reduced species with a clear isosbestic point at 344 nm as the concentration of $Ca(ClO_4)_2$ is raised. From such a spectral change, one can obtain the 1 : 1 complex formation constant K as 1.0×10^2 mol^{-1} dm^3 using the relation $(A - A_0)/(A_\infty - A) = K[Ca^{2+}]$, where A_0 is the absorbance of H_2L^1 itself and A_∞ is the absorbance of the Ca^{2+} complex. Essentially the same spectral change was observed for 4-methoxy-substituted derivative HL^2 in the titration with $Ca(ClO_4)_2$ in deaerated MeCN, from which the K value was determined as 1.1×10^2 dm^3 mol^{-1}.

Since the direct reaction with O_2 to give a covalent bond is spin forbidden, it has been proposed that the reaction of 1,5-dihydroflavin with O_2 occurs *via* an electron-transfer mechanism, resulting in a radical-ion pair, which collapses to form the covalent flavin-4*a*-hydroperoxide after spin conversion.[7,8] In this context, we have recently demonstrated that Mg^{2+} catalyses the electron transfer step from 1,5-dihydroflavin anion to O_2 *via* the complex formation of $O_2^{\cdot-}$ and Mg^{2+} to accelerate the overall two-electron oxidation of 1,5-dihydroflavin anion.[9] Thus, Ca^{2+} may also catalyse the electron transfer step from H_2L^1 to O_2. The contribution of the second-order dependence of k_{obs} on $[Ca^{2+}]$ in Fig. 2 indicates that the H_2L^1–Ca^{2+} complex is a reactive electron donor rather than free H_2L^1 as shown in Scheme 1. The electron transfer from H_2L^1–Ca^{2+} to O_2 is catalysed by Ca^{2+} *via* the complex formation of $O_2^{\cdot-}$ and Ca^{2+} as in the case of the Mg^{2+}-catalysed oxidation of 1,5-dihydroflavin anion.[9] The electron transfer may be accompanied with deprotonation and followed by fast hydrogen atom transfer to yield H_2O_2.

According to Scheme 1, the dependence of k_{obs} on $[Ca^{2+}]$ is derived as $k_{obs} = kK[Ca^{2+}]^2[O_2]/(1 + K[Ca^{2+}])$, where K is the complex formation constant of H_2L^1 with Ca^{2+} and k is the rate constant of the Ca^{2+}-catalysed oxidation of the reduced form by O_2 *via* formation of 1 : 1 complex between $O_2^{\cdot-}$ and Ca^{2+}. The kinetic equation can be rewritten as $[Ca^{2+}]k_{obs}^{-1} = (k[O_2])^{-1}[1 + (K[Ca^{2+}])^{-1}]$, where $[Ca^{2+}]k_{obs}^{-1}$ is linearly related to $[Ca^{2+}]^{-1}$. A linear plot of $[Ca^{2+}]k_{obs}^{-1}$ *vs.* $[Ca^{2+}]^{-1}$ is shown in the inset of Fig. 2. From the slope and intercept are obtained the rate constant $k = 8.6 \times 10^2$ dm^3 mol^{-1} s^{-1} and the complex formation constant $K = 1.0 \times 10^2$ dm^3 mol^{-1}. The K value thus obtained agrees perfectly with the K value (1.0×10^2 dm^3 mol^{-1}) determined independently from the titration. Such an excellent agreement supports the validity of Scheme 1, where the H_2L^1–Ca^{2+} complex is a reactive species for the oxidation by O_2. The spectral changes due to the formation of the H_2L^1–Ca^{2+} and HL^2–Ca^{2+} complexes are similar to that observed in the pH titration of $PQQH_2$ to $PQQH^-$.[6] Since the K value of H_2L^1 is about the same as that of HL^2 (*vide supra*), the binding position of Ca^{2+} with H_2L^1 and HL^2 may be the same. Thus, the coordination of Ca^{2+} may occur to the hydroxy oxygen at the 5 position as well as the pyridine nitrogen at the 6 position, facilitating the deprotonation of C(5)–OH proton to produce a much stronger electron donor than free H_2L^1. This may be the reason why the H_2L^1–Ca^{2+} complex acts as a reactive electron donor in the Ca^{2+}-catalysed electron transfer to O_2 (Scheme 1).



Scheme 1

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Footnote

† The presence of Ca^{2+} in the enzyme active site has been also suggested for other PQQ-dependent enzymes such as ethanol dehydrogenase from *Pseudomonas aeruginosa* and glucose dehydrogenase from *Acinetobacter calcoaceticus*, see ref. 10.

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