Is the calcium-ion catalysis of biological reoxidation of reduced PQQ purely electrostatic?†

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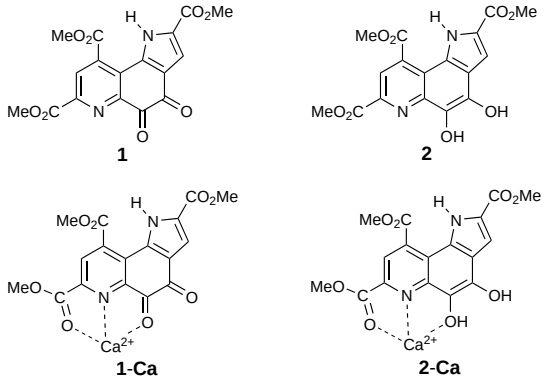
Semiempirical (PM3) molecular orbital calculations on the mechanism of reoxidation of PQQ by triplet dioxygen suggest that the reaction can proceed purely by hydrogen-atom-transfer steps that are catalysed by the electrostatic effect of a coordinated calcium ion, rather than by an electron-transfer mechanism.

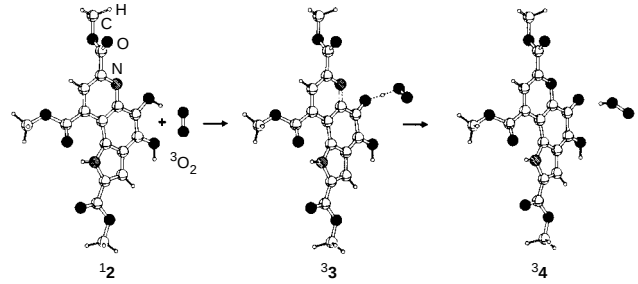
Pyrroloquinolinequinone (PQQ) **1** acts as the coenzyme in several bacterial oxidations.[1–3] It has been proposed[1–3] that the reoxidation of reduced PQQ ($PQQH_2$ **2**) is Ca-ion dependent.



Itoh *et al.*[4] recently demonstrated the catalytic effect of Ca^{2+} on the oxidation of **2** in MeCN solution by triplet O_2. They proposed an electron-transfer mechanism on the basis of earlier suggestions[5,6] that the reaction of 1,5-dihydroflavin with $^3O_2$ must occur by a single electron-transfer (SET) mechanism. However, reaction of **2** with $^3O_2$ to give a triplet radical pair is a perfectly spin-allowed reaction and the electrostatic effect of an adjacent positive charge should be to bind electrons tighter than in the neutral compound. Furthermore, we have pointed out[7–9] that reactions of closed shell molecules with $^3O_2$ should be strongly favoured by complexation with metal cations. We now suggest that the role of Ca^{2+} in the biological reoxidation of reduced PQQ is that of an electrostatic catalyst,[9] and that the Ca-dependent enzymes that use PQQ as a coenzyme are possibly the best documented examples of this type of catalysis in biological systems.

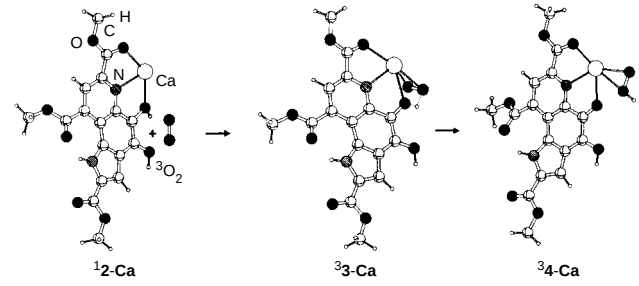
The reaction system **2** + $^3O_2 \rightarrow$ **1** + H_2O_2 with and without complexation to Ca^{2+} was investigated using standard PM3 semiempirical MO theory[10] with VAMP6.5.[11] The unrestricted Hartree–Fock (UHF) formalism was used throughout (for both singlets and triplets) and relative energies of different spin states were also checked using configuration interaction (CI) calculations. The Ca PM3 parameters are those developed by Hehre *et al.*[12] and made available *via* the Wavefunction website. All stationary points were characterised by calculation of their normal vibrations and the reaction paths were checked by internal reaction coordinate (IRC) calculations.

The calculations were carried out with the conformations shown in the Schemes. Complexation with Ca^{2+} gives structures **1-Ca** and **2-Ca**, as found in X-ray structures of bacterial methanol dehydrogenase.[13,14] Abstraction of the OH hydrogen on C-5 in ^{1}**2** by $^3O_2$ occurs *via* transition state ^{3}**3** to give ^{3}**4** (Scheme 1; superscripts before structure numbers indicate the spin multiplicity) in the uncomplexed case with a calculated activation energy of 44.1 kcal mol^{-1} (1 cal = 4.184 J) in a reaction that is found to be endothermic by 12.1 kcal mol^{-1}, neither thermodynamically nor kinetically favourable enough to occur at room temperature in an enzymatic system.



Scheme 1

The Ca-catalysed reaction **2-Ca** + $^3O_2 \rightarrow$ **4-Ca** *via* transition state **3-Ca** (Scheme 2), in contrast, requires an activation energy of only 3.9 kcal mol^{-1} and is exothermic by 33.2 kcal mol^{-1} and thus very favourable under biological conditions. Hence, both the kinetic stabilisation of the transition state arising from the charge effect on odd-electron bonds[15–18] and the thermodynamic effect of adding polar bonds to oxygen[7–9] are very effective in accelerating the reaction.

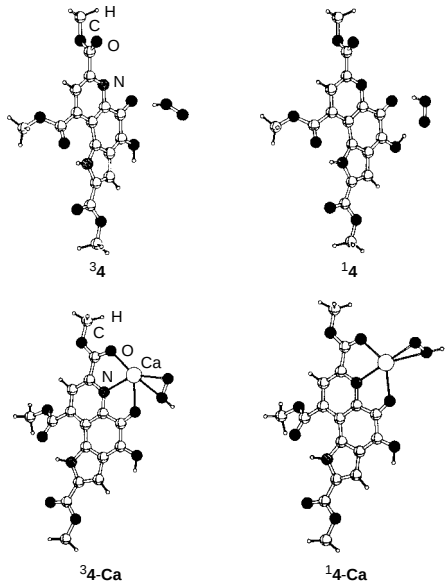


Scheme 2

Both single point CI calculations and UHF geometry optimisations of the singlet radical pairs corresponding to ^{3}**4** (^{1}**4** and ^{1}**4-Ca**) give energies that are almost degenerate with the triplet radical pairs. After geometry optimisation, ^{1}**4-Ca** is found to be 2.7 kcal mol^{-1} more stable than ^{3}**4-Ca**, so that intersystem crossing (ISC) to the singlet state should be fast, especially given the presence of the calcium ion and the oxygen centres.

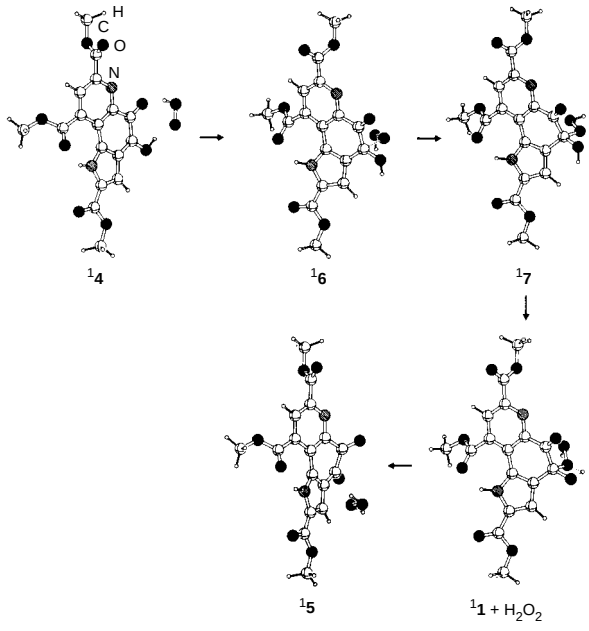
After ISC, the system can transfer the second OH hydrogen to the hydroperoxy radical to give the product. In the uncomplexed system, however, this reaction occurs in two steps, as shown in Scheme 3 and Fig. 1.

In contrast, the Ca-catalysed reaction proceeds directly *via* transition state ^{1}**5-Ca** to the final product ^{1}**1-Ca** + $^3O_2$ in a

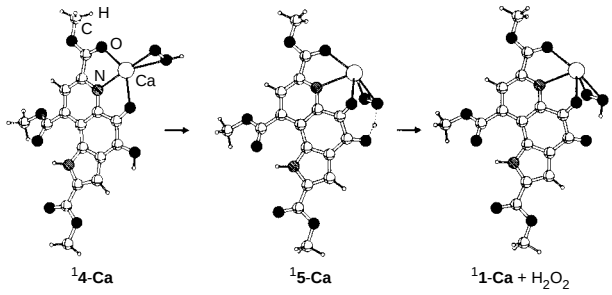


classical H-atom transfer step (Scheme 4). This reaction is calculated to have a barrier of 15.0 kcal mol^{-1}. The product is 55.2 and 19.3 kcal mol^{-1} more stable than ^{1}**4-Ca** and ^{1}**2-Ca** + $^3O_2$, respectively. The energy profiles for the two reaction paths are shown schematically in Fig. 1.

Although the SET mechanism has not been tested specifically, we believe that our calculations provide a strong indication that the role of the Ca ions in these systems is that of an electrostatic catalyst, as proposed in our model studies on



Scheme 3



Scheme 4

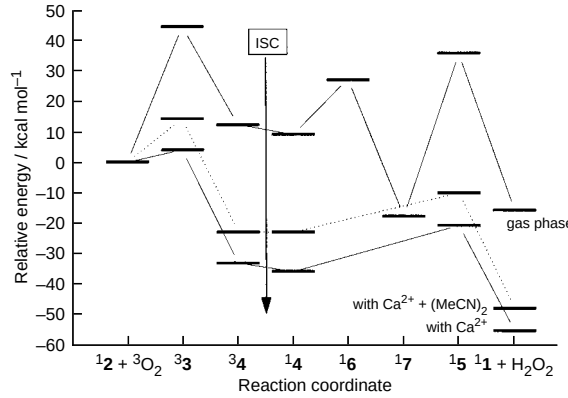


Fig. 1

methane oxidation and ethene epoxidation.[7–9] The PQQ-dependent systems therefore represent experimental examples of an effect first pointed out on the basis of model *ab initio* calculations.[7–9] We note especially that the direct formation of a covalent bond in such systems is not only spin-allowed, but is also a very facile process. There is no need to invoke an SET mechanism, especially in view of the likely fast ISC in **4-Ca**.

In order to investigate the effect of solvent, which would compete with oxygen for the complexation sites on the metal ion, we added two molecules of MeCN to the Ca-catalysed system. The energetic results are shown in Fig. 1. Although the effect of the metal is weakened somewhat, the total activation energy (14.1 kcal mol^{-1}) remains low enough for a very fast reaction at room temp.

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Footnotes and References

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