

The Role of Oxygen in the Mechanism of the Intramolecular Photoredox Reaction of Fe^{III} Protoporphyrin IX in Alkaline Aqueous Ethanol

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The evidence of superoxide formation during the irradiation of Fe^{III} protoporphyrin IX in oxygenated alkaline aqueous ethanol and the very short re-oxidation time of the photoredox Fe^{II} porphyrin product are consistent with a photoinduced electron transfer from ethanol to oxygen occurring with the assistance of an Fe^{III} porphyrin ethanolate complex.

The interest in the formation of superoxide in biological aerobic processes is on the increase in connection with the auto-oxidation of dioxygen carrier hemoproteins as well as with the toxic effect on living systems. In recent years, a number of very interesting studies on both the mechanism of superoxide formation and its reactivity with respect to biological substrates have been reported.¹

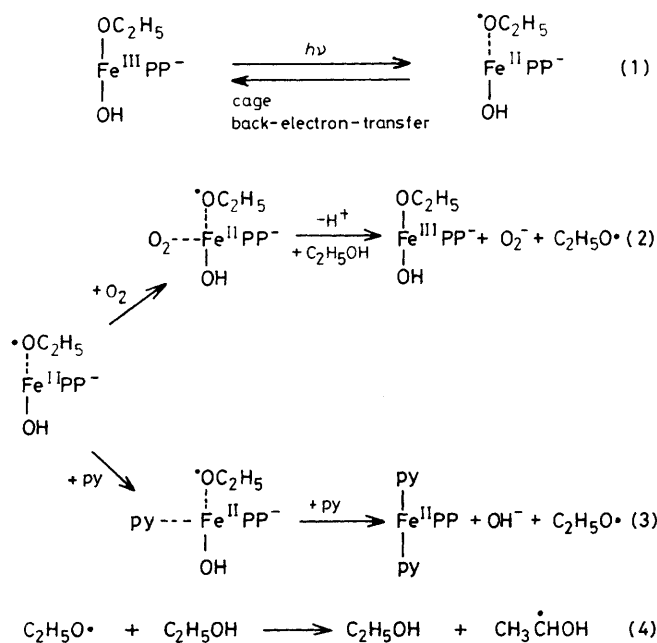
Fe^{II} porphyrin complexes have been extensively studied as they mimic the behaviour of hemoglobin as a dioxygen carrier. Despite the fact that the dioxygen carrying ability is known to be strongly inhibited in pathological conditions by the auto-oxidation of Fe^{II}, this process has not been sufficiently studied from kinetic and mechanistic points of view.

On the basis of the results obtained in a recently published work,² we report here an investigation on the photoredox reaction of Fe^{III} protoporphyrin IX in oxygenated aqueous ethanol in which evidence is given for the formation of superoxide ion and details of the reaction mechanism are discussed.

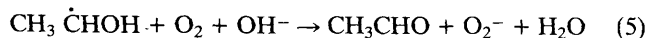
Fe^{III} protoporphyrin chloride (Fe^{III}PPCl) from Fluka was recrystallised following the reported glacial acetic acid method.³ Solutions were prepared by dissolving Fe^{III}PPCl in two types of solvents: 18% pyridine–40% ethanol–42% water (PEW) and 40% ethanol–60% water (EW). All the solutions were adjusted to pH 11 with NaOH and used within 24 h. No appreciable amounts of iron porphyrin dimeric species were observed to be formed in these conditions. The irradiation equipment was as previously reported.² E.s.r. measurements were carried out using a Bruker 220 SE spectrometer which was calibrated using α, α' -diphenylpicrylhydrazyl (DPPH). Laser-flash photolysis experiments were carried out using an Applied Photophysics detection system coupled with a J K system 2000 frequency-doubled ruby laser source, delivering 25 ns (half-width) pulses of 347 nm radiation. Transient decay

traces were stored and read on a Tektronix 7834 oscilloscope. Before each experimental run oxygenated and deaerated solutions were prepared by bubbling oxygen or nitrogen, respectively, into the reaction vessel through a glass capillary.

Fe^{III}PPCl solutions (1×10^{-3} mol dm⁻³ in solvent EW) were irradiated in an oxygen atmosphere at room temperature inside the e.s.r. spectrometer cavity with light of $\lambda > 305$ nm. After 10 minutes of irradiation the solutions were cooled to 95 K. Under these conditions an e.s.r. spectrum, consisting of an



asymmetric singlet ($g_{\parallel} = 2.055$ and $g_{\perp} = 2.004$), which is typical of a superoxide radical,⁴ was obtained. This represents the first experimental evidence of superoxide formation upon irradiation of an iron porphyrin complex. As expected, no evidence of superoxide was obtained with oxygen-free solutions.



In previous work we reported results obtained by flash-photolysis experiments on solutions of $\text{Fe}^{\text{III}}\text{PPCl}$.⁵ Since the flash-equipment used allowed only the detection of processes occurring in more than 30 μs , we have repeated the experiments with laser-flash equipment (see above for experimental details) which has a nanosecond time scale capability. The results obtained have shown that in PEW solvent the primary photoproduct $\text{Fe}^{\text{II}}\text{PP}$ is formed in less than 30 ns. In EW solvent no evidence of Fe^{II} porphyrin species is obtained on the same time scale. On the other hand, evidence for the formation of $\text{Fe}^{\text{II}}\text{PP}$ in oxygenated EW solutions has been previously obtained by e.s.r. spin trapping experiments showing the diffusion of an ethoxy radical away from the iron co-ordination sphere.²

The above results indicate that the primary photoredox products, *i.e.*, Fe^{II} porphyrin and ethoxy radical, revert to the initial ethanolate Fe^{III} porphyrin complex *via* a fast back-electron-transfer inside the solvent cage, unless species such as oxygen or pyridine are present which are capable of reacting with the Fe^{II} porphyrin intermediate when this is in some way bound to the ethoxy radical. The possible involvement of intermediates of this type was previously taken into account by other authors in a recent investigation on anion-promoted auto-oxidation processes of hemoglobins and myoglobins.⁶ The reaction mechanism is represented in equations 1–4 (py = pyridine).

The fact that in the oxygenated EW solvent the Fe^{II} re-oxidation occurs in a very short time (<30 ns) is a clear indication that oxygen indeed reacts with a co-ordinatively unsaturated Fe^{II} porphyrin intermediate before this intermediate can be stabilized by axial co-ordination.

The ethoxy radical in the bulk solution is rapidly converted into the hydroxyethyl radical by the excess of ethanol (reaction 4).⁷ The ethyl radical can undergo oxidation to acetaldehyde *via* a number of secondary reactions, one of which (equation 5) represents a method of superoxide radical formation.⁸ This reaction is known to be very efficient and is likely to occur in parallel with reaction 2.

A peculiar aspect of the postulated mechanism is that in the absence of pyridine the initial Fe^{III} protoporphyrin ethanolate complex is found unmodified at the end of the reaction sequence. This implies that in the overall process an oxygen reduction by ethanol takes place with the photo-assistance of an Fe^{III} protoporphyrin complex. It should be noticed, however, that prolonged irradiations caused an absorbance decrease in the whole u.v.–visible spectral range scanned (300–800 nm). This indicates an irreversible modification of the Fe^{III} porphyrin complex, which can be attributed to the ring opening.

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