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A kinetic study of the formation of a model high-valent diiron non-heme complex, $[Fe^{III}Fe^{IV}(\mu\text{-}O)_2(tpa)_2]^{3+}$ (tpa = tris(2-pyridylmethyl)amine), by cryogenic stopped-flow techniques†

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$[Fe_2(\mu\text{-}O)(tpa)_2(OH)(H_2O)]^{3+}$ reacts with H_2O_2 in acetonitrile at −40 °C forming a transient diferric peroxo intermediate (λ_{max} = 700 nm, ε = 1800 dm^3 mol^{-1} cm^{-1}), which then reacts with a second H_2O_2 molecule to give $[Fe^{III}Fe^{IV}(\mu\text{-}O)_2(tpa)_2]^{3+}$.

Non-heme diiron redox enzymes have been one of the "hot topics" of bioinorganic chemistry for the past decade. Their common mechanistic feature is the formation of a diiron(III) peroxo complex, which then transforms into a high-valent diiron species responsible for the oxidative action of an enzyme.[1–4] The actual need for modeling such a process on simple diiron complexes has been recently stated.[1,2] The only well characterized structural and functional models for the high-valent diiron species are $Fe^{III}Fe^{IV}(\mu\text{-}O)_2$ diamond core complexes with tpa and its derivatives, formed from corresponding Fe^{III}_2-complexes and H_2O_2 at low temperature.[2,5–7] A relatively long-lived peroxo complex has been observed as an intermediate in the formation of $Fe^{III}Fe^{IV}(\mu\text{-}O)_2(6\text{-}Me_3\text{-}tpa)_2{}^{3+}$ (6-Me$_3$-tpa = tris(6-methyl-2-pyridylmethyl)amine),[8] with the general scheme similar to the peroxide shunt of diiron non-heme enzymes.[1–3] In this paper, we communicate the direct observation of a transient model diferric peroxo complex quickly converting into a high-valent species.

Mixing of $[Fe^{III}_2(\mu\text{-}O)(tpa)_2(OH)(H_2O)]^{3+}$ and H_2O_2 solutions in acetonitrile at −40.0 ± 0.1 °C under pseudo-first order conditions (10-fold or larger excess of H_2O_2) produces kinetic traces (Fig. 1), which are well fit by two exponential functions yielding wavelength-independent sequential rate constants k'_{obs} and k''_{obs}.‡ Treatment of the data by IS-2 Rapid Kinetics Software (Hi-Tech) using the A→B→C model gave electronic spectra of the three species involved (Fig. 1). The initial and final spectra agree well with those reported for $[Fe^{III}_2(\mu\text{-}O)(tpa)_2(OH)(H_2O)]^{3+}$ and $[Fe^{III}Fe^{IV}(\mu\text{-}O)_2(tpa)_2]^{3+}$, correspondingly.[5,7] The spectrum of the intermediate $B_{(peroxo)}$ has a broad maximum at about 700 nm (ε = 1800 dm^3 mol^{-1} cm^{-1}), which is very similar to the H_{peroxo} intermediate of methane monooxygenase (λ_{max} = 700 nm, ε = 1800 dm^3 mol^{-1} cm^{-1})[4] and the peroxo intermediate of a mutant ribonucleotide reductase (λ_{max} = 700 nm, ε = 1500 dm^3 mol^{-1} cm^{-1}).[9] Such a band is characteristic of $Fe^{III}_2(\mu\text{-peroxo})$ complexes[10,11] and is referred to LMCT.[12] A similar $Fe^{III}_2(\mu\text{-}O)(\mu\text{-}O_2)(6\text{-}Me_3\text{-}tpa)_2{}^{2+}$ complex has been reported, which converts into $[Fe^{III}Fe^{IV}(\mu\text{-}O)_2(6\text{-}Me_3\text{-}tpa)_2]^{3+}$ upon the addition of one equiv. of acid.[8] Thus, the species B_{peroxo} is most probably $[Fe^{III}_2(\mu\text{-}OH)(\mu\text{-}O_2)(tpa)_2]^{3+}$ or $[Fe^{III}_2(\mu\text{-}O_2)(tpa)_2(OH)(H_2O)]^{3+}$. It can be also a mixture of these and other diferric peroxo complexes.[10] The rate constant k'_{obs} corresponds to the formation of B_{peroxo}, and k''_{obs} to the transformation of B_{peroxo} into the $Fe^{III}Fe^{IV}$-species. Concentration dependences for the constants k'_{obs} and k''_{obs} allow us to propose a mechanistic scheme for these two consecutive steps (Scheme 1).

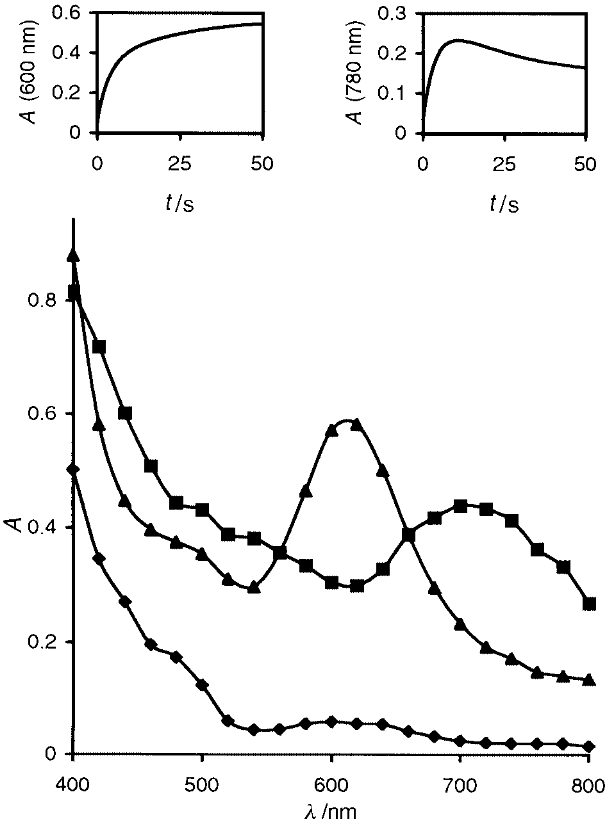
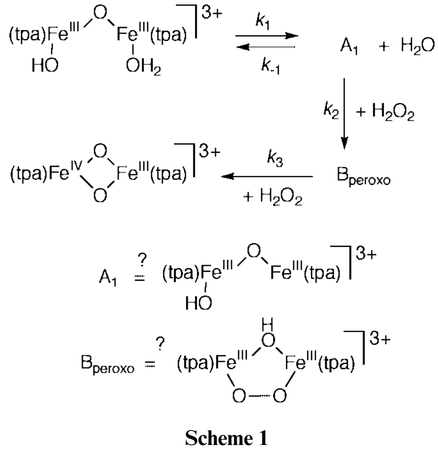


Fig. 1 Spectra of the initial complex $[Fe_2(\mu\text{-}O)(tpa)_2(OH)(H_2O)]^{3+}$ (2.5 × 10^{-4} mol dm^{-3}) (◆), transient species B_{peroxo} (■) and $[Fe^{III}Fe^{IV}(\mu\text{-}O)_2(tpa)_2]^{3+}$ (▲) calculated from kinetic data. $[H_2O_2]_o$ = 2.5 × 10^{-3} mol dm^3 (after mixing).



Scheme 1

The formation of the transient intermediate B_{peroxo} is decelerated by water and accelerated by hydrogen peroxide. Plots of kinetic data $k'_{obs}{}^{-1}$ *vs.* $[H_2O]$ at constant $[H_2O_2]$ and $k'_{obs}{}^{-1}$ *vs.*

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† *Supplementary data available*: plots of kinetic data. For direct electronic access see http://www.rsc.org/suppdata/dt/1999/3335/, otherwise available from BLDSC (No. SUP 57624, 4 pp.) or the RSC Library. See Instructions for Authors, 1999, Issue 1 (http://www.rsc.org/dalton).

$[H_2O_2]^{-1}$ at constant $[H_2O]$ are straight lines with practically the same intercept. (SUP 57624) Such behavior suggests the formation of another intermediate (A_1), which exists in equilibrium with the starting complex $[Fe^{III}_2(\mu\text{-}O)(tpa)_2(OH)(H_2O)]^{3+}$ and converts irreversibly into B_{peroxo}. Spectral kinetic data (Fig. 1) show that the intermediate A_1 does not accumulate in the reaction mixture to an appreciable amount, and that the reaction $[Fe^{III}_2(\mu\text{-}O)(tpa)_2(OH)(H_2O)]^{3+} \rightarrow A_1$ is the rate-limiting step.

The simplest model to account for the observations is shown in Scheme 1.§

$$k'_{obs} = \frac{k_1(k_2/k_{-1})[H_2O_2]}{(k_2/k_{-1})[H_2O_2] + [H_2O]}$$

The constant $k_1 = 0.5 \pm 0.1\ s^{-1}$ corresponds to the rate of the H_2O molecule dissociation in $[Fe^{III}_2(\mu\text{-}O)(tpa)_2(OH)(H_2O)]^{3+}$. The ratio $k_2/k_{-1} = 6 \pm 2$ reflects the larger nucleophilicity of H_2O_2 compared to H_2O (α-effect).[13] The dissociative mechanism is common for Fe^{III} olated aqua species due to the labilizing effect of the OH^- ligand.[14] A similar action can be expected from the O^{2-} ligand. Thus, the intermediate A_1 probably is $[Fe^{III}_2(\mu\text{-}O)(tpa)_2(\eta^1\text{-}OH)]^{3+}$ or $[Fe^{III}_2(\mu\text{-}O)(\mu\text{-}OH)(tpa)_2]^{3+}$.[15] It can react with H_2O_2 to form $[Fe^{III}_2(\mu\text{-}O)(tpa)_2(OH)(H_2O_2)]^{3+}$, which then converts into $[Fe^{III}_2(\mu\text{-}O_2)(\mu\text{-}OH)(tpa)_2]^{3+}$ or $[Fe^{III}_2(\mu\text{-}O_2)(tpa)_2(OH)(H_2O)]^{3+}$ by proton transfers. The apparent ε of B_{peroxo} (calculated from kinetic data) does not significantly depend on water concentration up to $[H_2O]/[H_2O_2] = 40$. It confirms the presence of a practically irreversible stage in the formation of the peroxo intermediate. Peroxide is apparently a much better ligand for Fe^{III} than oxide or hydroxide, as stable $Fe^{III}(O_2^{2-})$-complexes can form even in aqueous solution.[16]

The observed pseudo-first order rate constant (k''_{obs}) of the following transformation $B_{peroxo} \rightarrow [Fe^{III}Fe^{IV}(\mu\text{-}O)_2(tpa)_2]^{3+}$ does not depend on $[H_2O]$, but is proportional to $[H_2O_2]$.

$$k''_{obs} = k_3[H_2O_2]$$

The graph k''_{obs} *vs.* $[H_2O_2]$ is a straight line with a practically zero intercept and a slope of $k_3 = 10 \pm 2\ dm^3\ mol^{-1}\ s^{-1}$, corresponding to a second-order rate constant for the reaction between B_{peroxo} and H_2O_2 (SUP 57624). The reaction is apparently a redox process with tentative overall stoichiometry:

$$2[Fe^{III}_2(\mu\text{-}O_2)(\mu\text{-}OH)(tpa)_2]^{3+} + H_2O_2 \longrightarrow 2[Fe^{III}Fe^{IV}(\mu\text{-}O)_2(tpa)_2]^{3+} + O_2 + 2H_2O$$

It should be noted that the presence of a reductant is essential for the action of native ribonucleotide reductase, presumably in order to reduce the Fe^{III}_2-peroxo intermediate to the high-valent $Fe^{III}Fe^{IV}$ intermediate X.[3] In the model system studied here, H_2O_2 is the most probable reductant.

The yields of $[Fe^{III}Fe^{IV}(\mu\text{-}O)_2(tpa)_2]^{3+}$ have never been quantitative, reaching the maximum of *ca.* 70% (based on the reported value $\varepsilon = 5500\ dm^3\ mol^{-1}\ cm^{-1}$)[6] at 40-fold or more excess of H_2O_2, which corresponds to $[H_2O_2]_o \geq 10$ mM under our conditions. The same maximum yield has been reported by Que's group with only 1.5-fold excess of H_2O_2, which corresponds also to *ca.* 10 mM H_2O_2.[7] These data can be rationalized by invoking an independent pathway of B_{peroxo} decomposition, which plays a larger role at insufficient absolute (rather than relative) H_2O_2 concentrations.

At room temperature the $[Fe_2(\mu\text{-}O)(tpa)_2(OH)(H_2O)]^{3+}$ complex causes intense effervescence of the H_2O_2 acetonitrile solution. Significant catalatic activity has been reported for $[Fe_2(\mu\text{-}O)(\mu\text{-}O_2CCH_3)(tpa)_2]^{2+}$ in such conditions.[17] To determine, if the excess of H_2O_2 survives at low temperature, additional double-mixing experiments were carried out. The 5 mM H_2O_2 solution was first mixed with 0.5 mM $[Fe_2(\mu\text{-}O)(tpa)_2(OH)(H_2O)]^{3+}$ solution and then, upon the completion of apparent spectral changes, with a fresh portion of 0.25 mM $[Fe_2(\mu\text{-}O)(tpa)_2(OH)(H_2O)]^{3+}$ solution. Kinetic quantitation showed that no more than 2 moles of H_2O_2 per mole of the Fe_2-complex had been consumed in the first mixing. Thus, the catalatic process in this system is negligible and suppressed at −40 °C.

In the other series of double-mixing experiments the $[Fe_2(\mu\text{-}O)(tpa)_2(OH)(H_2O)]^{3+}$ solution was first mixed with H_2O_2 solution, and upon the completion of apparent spectral changes, with a fresh portion of more concentrated H_2O_2 solution. It was found that the ratio $H_2O_2 : Fe_2 \geq 1.5 \pm 0.1$ was enough to consume all of the initial diiron complex and prevent the formation of new B_{peroxo} in the second mixing. This confirms the presence of an irreversible stage in the formation of B_{peroxo} and suggests that the starting complex $[Fe_2(\mu\text{-}O)(tpa)_2(OH)(H_2O)]^{3+}$ is not regenerated in the system. The stoichiometry of the initial interaction between $[Fe_2(\mu\text{-}O)(tpa)_2(OH)(H_2O)]^{3+}$ and H_2O_2 to give B_{peroxo} is most probably 1 : 1, with some H_2O_2 consumed in the concomitant formation of the $Fe^{III}Fe^{IV}$ complex and in a minor catalatic process.

Other kinetic experiments regarding the formation and reactivity of $Fe_2O_2(H)$ diamond cores are currently under way.

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Notes and references

‡ The initial complex, $[Fe_2(\mu\text{-}O)(tpa)_2(OH)(H_2O)](ClO_4)_3$ was prepared by a published procedure.[5] Hydrogen peroxide, 30% aqueous solution (ACS certified grade) and acetonitrile (HPLS grade) were purchased from Fisher. Stopped-flow experiments were carried out by using a Hi-Tech Scientific (Salisbury, UK) SF-43 cryogenic stopped-flow apparatus with stainless steel plumbing. Measurements were made at wavelengths from 360 to 800 nm with 20 nm intervals to get spectral information.

§ More complicated molecular interpretations can also describe the observations. However, another simple model, with the fast preequilibrium $[Fe_2(\mu\text{-}O)(tpa)_2(OH)(H_2O)]^{3+} + H_2O_2 \rightleftharpoons A_1 + H_2O$ and the rate-limiting step $A_1 \rightarrow B_{peroxo}$, can be discounted. It implies that A_1 is an Fe^{III}-peroxo complex, which is formed in substantial amount in the reaction mixture (the preequilibrium constant $K_{eq} = 6 \pm 2$). Such an assumption contradicts the spectral changes calculated from kinetic data.

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