High Activity of an Fe–tfda (tfda = 2-aminomethyltetrahydrofuran-*N,N*-diacetic acid) Complex for Hydroxylation at the Aromatic and Alkane Rings of 2′-Deoxyguanosine in the Presence of Hydrogen Peroxide

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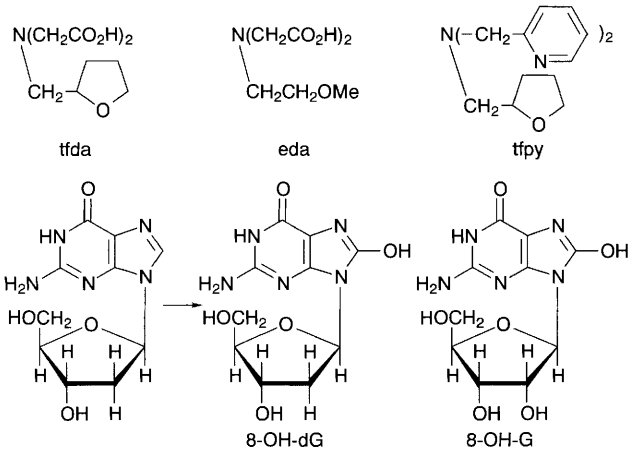
An iron(III) complex with tfda (2-aminomethyltetrahydrofuran-*N,N*-diacetic acid) exhibits high activity for hydroxylation at the 8 and 2′ positions of deoxyguanosine in the presence of hydrogen peroxide; this demonstrates that a facile transformation from deoxyribonucleotide to ribonucleotide *in vivo* may be possible.

Structural and alkane functionalization studies of plausible biomimetic models of methane monooxygenase are an active area of research.[1,2] Recent spectroscopic studies, including an X-ray crystallographic analysis on methane monooxygenase enzyme (MMO) have shown that the active site has a diiron μ-hydroxo structure, Fe_2(μ-hydroxo), with both terminal and μ-carboxylate anions and a terminal H_2O ligand as well as terminal histidine ligands.[3] Until now, several binuclear iron(III) compounds with μ-oxo bridges have been used as a model for MMO. In this study, we have found that a binuclear iron(III) complex with tfda (tfda = 2-aminomethyltetrahydrofuran-*N,N*-diacetic acid) exhibits abnormally high activity in the hydroxylation reactions of both the aromatic and alkane rings of 2′-deoxyguanosine in the presence of hydrogen peroxide (Scheme 1).

The iron(III) complex solution with tfda was prepared as follows. The pH of an aqueous solution containing ferric chloride and tfda (Fe^{III} : tfda = 1 : 2 molar ratio) was adjusted to 7.0 by the addition of $KHCO_3$; the solution was green and the absorption spectrum of the solution indicated the presence of a binuclear iron(III) species with μ-oxo-μ-carbonato bridges, as exemplified by analogous compounds.[4,5] The absorption bands observed in the visible region, which are characteristic for binuclear iron(III) compounds with oxo and carbonato bridges, disappeared upon the addition of hydrogen peroxide (tenfold excess; the solution also turned red) and re-appeared with the decomposition of the hydrogen peroxide added. This indicates that coordination of hydrogen peroxide to an iron(III) atom occurs in the solution, and that the formation of the peroxide adduct is reversible. The red species formed by the coordination of hydrogen peroxide may be an iron(III) compound with a mono (μ-oxo) bridge, because the absorption spectrum of the red species is very similar to those of the analogous compounds.[6]

In Fig. 1, the HPLC† of the solution (pH adjusted to 7.0 by addition of $KHCO_3$) containing $FeCl_3 \cdot 6H_2O$, tfda, 2′-deoxyguanosine, and hydrogen peroxide is illustrated [(*c*) and (*d*)]. It should be noted here that the signal intensity corresponding to 8-hydroxydeoxyguanosine (8-OH-dG; indicated by an arrow in Fig. 1 at a retention time of 5.8 min)[7] is much larger than that of other iron(III) compounds such as Fe–edda (edda = ethylenediamine-*N,N′*-diacetic acid) and Fe–eda [see Fig. 1(*a*) and (*b*)]. This demonstrates that Fe–tfda exhibits abnormally high activity for the hydroxylation of the 8 position of 2′-deoxyguanosine in the presence of hydrogen peroxide. In addition to this signal, another new signal at a retention time of 5.0 min [see Fig. 1(*c*) and (*d*)] was observed only for the Fe–tfda system. The intensity of this signal also increases with time, as shown. In order to obtain information about this compound, we investigated the reaction between Fe–tfda and guanosine in the presence of hydrogen peroxide. Although other iron(III) complexes used in this study cannot form 8-OH-guanosine (8-OH-G) in the presence of hydrogen peroxide, the Fe–tfda solution can also catalyse the formation of 8-OH-G (*ca.* 4% based on deoxyguanosine added) under the same experimental conditions (not shown). The signal position of the 8-OH-G was identified by the use of an authentic sample.[7] This study clearly



Scheme 1

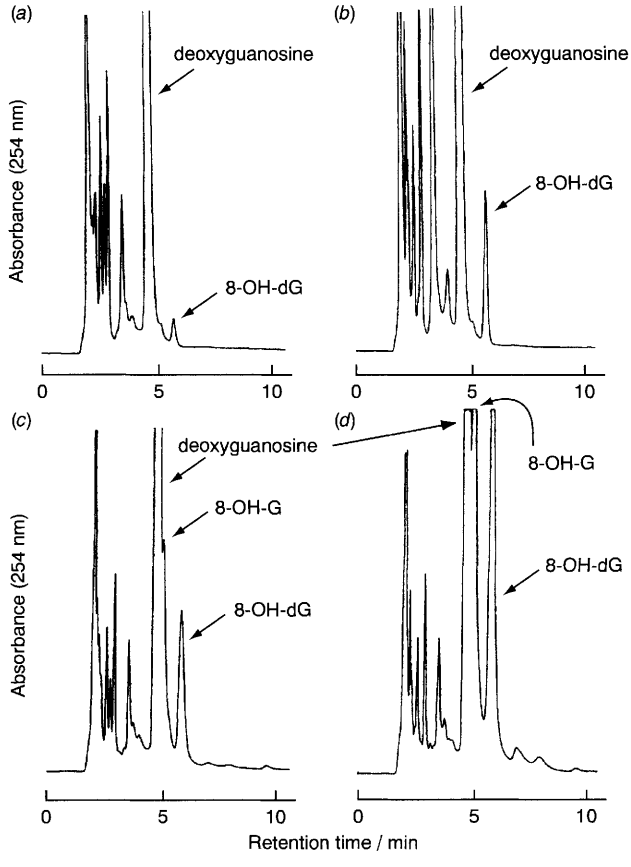
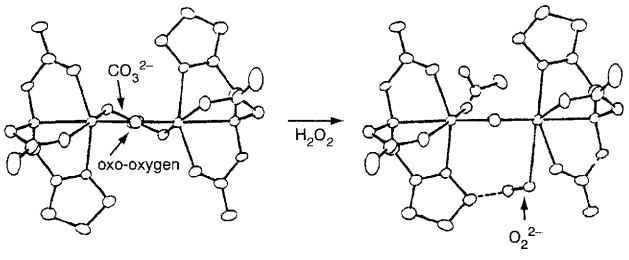


Fig. 1 HPLC of a solution containing iron(III) complex, deoxyguanosine, and hydrogen peroxide.† (*a*) Fe^{III}–eda solution, 1 min after addition of hydrogen peroxide; (*b*) Fe^{III}–eda solution, 30 min after addition; (*c*) Fe^{III}–tfda solution, 1 min after addition of hydrogen peroxide; (*d*) Fe^{III}–tfda solution, 15 min after addition. (Other main peaks are: 2.0 min, base propenals; 2.9 min, guanine; 3.9 min, 8-OH-guanine).

revealed that the signal observed at 5.0 min in the mixture of Fe–tfda, deoxyguanosine and hydrogen peroxide is undoubtedly due to 8-OH-G.

The above facts imply that a hydroxylation reaction at the 2′ position of deoxyguanosine proceeds in the Fe–tfda and hydrogen peroxide system (see Scheme 1). We have examined this reaction using more than twenty iron(III) compounds; however, Fe–tfda is the only compound that can hydroxylate the 2′ position of deoxyguanosine in the presence of hydrogen peroxide. Very recently we have determined the crystal structure of the iron(III) complex with tfpy, $Fe_2OCl_2(tfpy)_2^{2+}$, the structure being almost the same as that of the (tpa) derivative,[8] and observed that the coordination ability of the oxygen atom of the tetrahydrofuran ring is comparable to that of the nitrogen atom of the pyridine ring.‡ Based on the above facts, we propose that at the first stage the coordination of hydrogen peroxide to an iron(III) atom occurs, and the coordinated hydrogen peroxide may attach to the carbon atom of the tetrahydrofuran ring, giving a metal–alkylhydroperoxide adduct (see Scheme 2; the structure of the iron(III)–tfda complex is assumed on the basis of the analogous iron(III)–pac complex[4]), which is supported by the fact that formation of a hydroperoxide adduct of cyclohexane was confirmed in the reaction mixture of iron(III) complex, hydrogen peroxide, and cyclohexane.[9] this metal–alkylhydroperoxide adduct may react with aromatic or alkane rings, yielding an oxygenated species. Although we have no direct evidence for the formation of a hydroperoxide adduct, the present system should give valuable information on the elucidation of the mechanism of oxygen activation in MMO.



Scheme 2

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Footnotes

† To an aqueous solution (50 ml; pH adjusted to 7.0 by addition of $KHCO_3$) containing iron(III) chloride hexahydrate (270 mg), tfda (440 mg), and deoxyguanosine (20 mg) was added 10 ml of hydrogen peroxide solution (1×10^{-1} mol dm^{-3}), and the resulting solution was evaluated in terms of HPLC at room temperature [column: Cosmosil $5C_{18}$-AR packed column, 4.6 mm i.d. × 150 mm (Waters); solvent[8]: 10% methanol–water, 10 mmol dm^{-3} ammonium acetate (pH = 5.3)].

‡ Crystal structure of $[Fe_2OCl_2(tfpy)_2][FeCl_4]_2$ was solved. Fe–O (ethereal oxygen of tetrahydrofuran ring) is 2.226 Å.

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