Synthesis, Structure, and Spectroscopic Properties of $[Fe^{III}(tnpa)(OH)(PhCOO)]ClO_4$: A Model Complex for an Active Form of Soybean Lipoxygenase-1**

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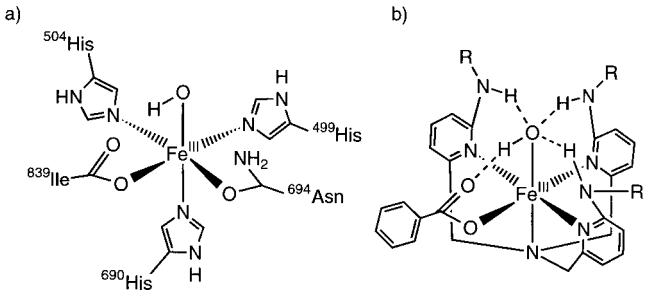
Lipoxygenases (LOs) are mononuclear non-heme iron enzymes that catalyze the peroxidation of polyunsaturated fatty acids and fatty acid esters containing the *cis,cis*-1,4-diene moiety to the corresponding 1-hydroperoxy-*trans,cis*-2,4-diene. The proposed active oxidizing species in the catalytic cycle of soybean lipoxygenase-1 (SLO-1) probably has a six-coordinate iron(III) core structure with three histidine imidazole units, one carboxylato ligand from the C-terminal isoleucine, one asparagine carboxamidato carbonyl oxygen atom, and one hydroxo ligand (Scheme 1 a).[1–5]



Scheme 1. a) A proposed (hydroxo)iron(III) core structure of the active species in the catalytic cycle of soybean lipoxygenase-1. Adapted from ref. [1 a] p. 285. b) The six-coordinate (hydroxo)iron(III) complex **1**. Dashed lines represent intramolecular hydrogen bonds. $R = CH_2C(CH_3)_3$.

Although structures of mononuclear six-coordinate iron(III) complexes with oxygen-donor ligands such as methoxo, CH_3OH, carboxylato, or carboxamidato, and dinuclear iron(III) complexes with terminal or bridging hydroxo ligands have been reported as models for the iron(III) form of SLO-1 (ferric SLO-1),[6] mononuclear six-coordinate (hydroxo)-iron(III) complexes have, until now, not been reported. Herein, we report the synthesis, structure, and spectroscopic properties of such a complex: $[Fe(tnpa)(OH)(PhCOO)]ClO_4$ (**1**, tnpa = tris(6-neopentylamino-2-pyridylmethyl)amine); this is the first structural model for the active center of ferric SLO-1 with a hydroxo ligand. Complex **1** has been characterized by X-ray structure analysis, UV/Vis, IR, and EPR spectroscopy, and electrospray ionization mass spectrometry (ESI-MS).

A new tetradentate tripodal ligand tnpa was designed and synthesized that would complex to an Fe^{III} center (Scheme 1 b) and mimic specific attributes of ferric SLO-1. The ligand would bind to the Fe^{III} center in a four-coordinate mode (by one tertiary amine and three pyridine units) leaving two vacant coordination sites on the metal center free to accommodate fifth and sixth exogenous (e.g. carboxylato and hydroxo) ligands. Complex **1** was synthesized by a reaction of $Fe(ClO_4)_3 \cdot x\,H_2O$ with an equimolar amount of tnpa and PhCOONa in acetonitrile containing a small amount of H_2O. To establish the origin of the hydroxo ligand in **1**, the same synthesis of **1** has also been carried out in acetonitrile containing a small amount of $H_2{}^{18}O$. ESI-MS results confirm that the labeled oxygen atom is incorporated into the hydroxo ligand. The IR spectrum of **1** shows the O–H vibration at 3277 cm^{-1} shifts to 3273 cm^{-1} when $H_2{}^{18}O$ is added.[7] Complex **1** is stable for months under air at ambient temperature.

Purple crystals of **1** used in the X-ray structure analysis (Figure 1) were obtained by diffusion of diethyl ether into a solution of **1** in acetonitrile.[8] All hydrogen atoms, which were located at the positions generated by Fourier-difference syntheses, were included in the least-squares calculation of **1**. The iron atom adopts a distorted octahedral coordination, it is surrounded by the tetradentate, tripodal tnpa and the monodentate $PhCOO^-$ and OH^- ligands. The Fe–OH bond length of 1.876(2) Å agrees well with that of ferric SLO-1 (1.88 Å) as established by EXAFS (extended X-ray absorp-

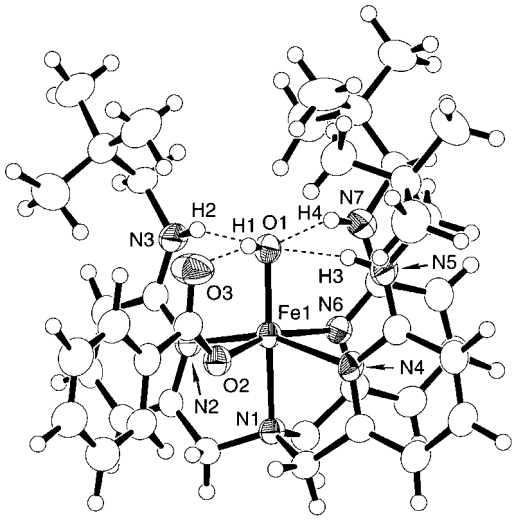


Figure 1. ORTEP drawing of **1**. Selected bond lengths [Å] and angles [°] as well as interatomic distances [Å]: Fe1–O1 1.876(2), Fe1–O2 1.988(2), Fe1–N1 2.158(3), Fe1–N2 2.183(2), Fe1–N4 2.182(2), Fe1–N6 2.228(3), O1–H1 0.83(3); O1-Fe1-N1 174.0(1), O2-Fe1-N6 164.9(1), N2-Fe1-N4 156.8(1), O1-Fe1-O2 97.2(1), O1-Fe1-N2 100.13(9), O1-Fe1-N4 103.04(9), O1-Fe1-N6 95.4(1), O2-Fe1-N1 88.2(1), O2-Fe1-N2 90.06(9), O2-Fe1-N4 88.83(9), N1-Fe1-N2 77.37(9), N1-Fe1-N4 79.39(9), N1-Fe1-N6 79.5(1), N2-Fe1-N6 95.86(9), N4-Fe1-N6 80.23(9), O1-H1-O3 157(4); O1–O3 2.680(3), O1–N3 2.857(3), O1–N5 2.894(3), O1–N7 2.835(3).

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tion fine structure) analysis.[3a] Complex **1** has a *cis* configuration between the OH^- ligand and the $PhCOO^-$ ligand as well as with the three NH ligands of tnpa, which enables the formation of four intramolecular hydrogen bonds (Figure 1; H1 – O3 = 1.89(4), H2 – O1 = 2.04(4), H3 – O1 = 2.08(4), and H4 – O1 = 2.03(3) Å). These hydrogen bonds may contribute to the stabilization of the (hydroxo)iron(III) core structure of **1**. Such stabilization by hydrogen bonds was also demonstrated in $[Cu(tppa)(OH)]ClO_4$ and $[Cu(bppa)(OOH)]ClO_4$[9] (tppa = tris(6-pivalamido-2-pyridylmethyl)amine; bppa = bis(2-pyridylmethyl)(6-pivalamido-2-pyridylmethyl)-amine). Moreover, the steric bulk of the neopentylamino groups of tnpa prevents the complex from polymerizing and thus these groups are required to form a stable (hydroxo)-iron(III) complex **1**.

The EPR spectrum of **1** in acetonitrile at 77 K is dominated by signals at $g = 5.16$ and 1.97, which are ascribed to a rhombic high-spin Fe^{III} center. Magnetic susceptibility measurements of **1** show $\mu_{eff} = 5.9\ \mu B$. (with an estimated maximal error of $\pm 10\%$) at 297 K, corresponding to a high-spin Fe^{III} atom ($S = 5/2$).[10] Complex **1** exhibits a quasi-reversible cyclic voltammetric wave in acetonitrile at ambient temperature with $E_{1/2} = -0.125$ V (vs. $Ag^+/AgCl$, $\Delta E = 100$ mV), which is assigned to the Fe^{3+}/Fe^{2+} couple. It was confirmed by ESI-MS that the structure of **1** is preserved in acetonitrile (Figure 2a).[11] The positive-ion mass spectrum of **1** in the range of *m/z* 100 to 2000 shows prominent signals at *m/z* 739.6 (relative intensity (I) = 100%) and *m/z* 617.4 (I = 70%). The signal at *m/z* 739.6 has a characteristic distribution of isotopomers that matches well with the calculated isotopic distribution for $[Fe(tnpa)(OH)(PhCOO)]^+$ ($[\mathbf{1} - ClO_4]^+$, Figure 2c, d). MS/MS measurements show that the signal at *m/z* 617.4 is a product ion ($[\mathbf{1} - ClO_4 - PhCOOH]^+$) of $[\mathbf{1} - ClO_4]^+$.

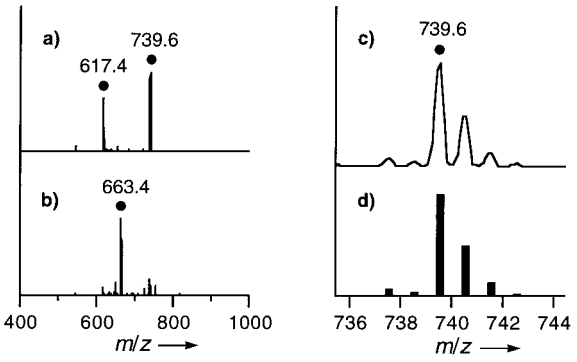

Figure 2. a) The positive-ion ESI mass spectrum of **1** in acetonitrile. The signal at *m/z* 739.6 corresponds to $[\mathbf{1} - ClO_4]^+$, that at *m/z* 617.4 is a product ion of $[\mathbf{1} - ClO_4]^+$. b) The positive ion ESI mass spectrum of **1** in methanol. Magnified representation of the signal at *m/z* 663.4 corresponds to $[\mathbf{2} - ClO_4]^+$. c) The signal at *m/z* 739.6. d) The calculated isotopic distribution for $[\mathbf{1} - ClO_4]^+$.

Complex **1** exhibits a UV/Vis spectrum with a distinct band at 536 nm ($\varepsilon = 1000\ M^{-1}cm^{-1}$) in acetonitrile (Figure 3). This observation is in contrast to reports of yellow ferric SLO-1 having its broad absorption band at 330 nm ($\varepsilon = 1600\ M^{-1}cm^{-1}$ in H_2O).[12] Interestingly, complex **1** gives a yellow-orange (dimethoxo)iron(III) complex $[Fe(tnpa)(OMe)_2]ClO_4$ (**2**) in methanol as evident from the UV/Vis (Figure 3) and ESI mass (Figure 2b) spectra. A prominent signal at *m/z* 663.4 (I = 100%) in the ESI mass spectrum corresponds to $[Fe(tnpa)(OMe)_2]^+$ ($[\mathbf{2} - ClO_4]^+$). Complex **2** was isolated as a yellow-orange powder from the solution of **1** in methanol.[13] Complex **2** exhibits an EPR signal at $g = 4.25$, indicating a rhombic high-spin Fe^{III} center. Scarrow et al. have indicated that the coordination in ferric SLO-1 is less affected by the presence of alcohols than the site in the iron(II) form of SLO-1 (ferrous SLO-1).[3a] However, the OH group in the non-heme iron complex **1** is readily substituted by methanol.

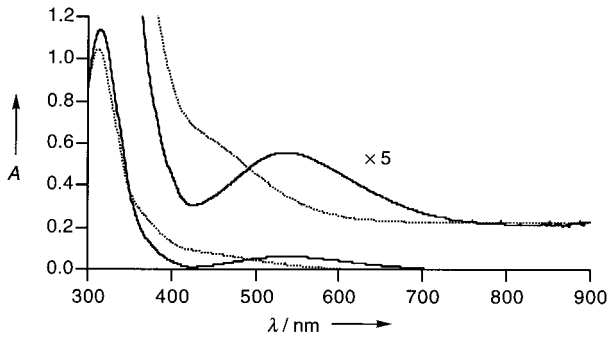

Figure 3. UV/Vis spectra of **1** in acetonitrile (solid line) and in methanol (dotted line).

In summary, a new six-coordinate (hydroxo)iron(III) complex **1** with four N-donor (one tertiary amino and three pyridyl) ligands and two O-donor (hydroxo and carboxylato) ligands has been prepared. These ligands are similar to those of the proposed active center of ferric SLO-1 with three N-donor (imidazolyl) ligands and three O-donor (hydroxo, carboxylato, and carboxamidato) ligands. Furthermore, complex **1** has a *cis* configuration between the OH^- ligand and the $PhCOO^-$ and the three NH ligands of tnpa. The proposed core structure of ferric SLO-1 also has a *cis* configuration between the hydroxo ligand and the carboxylato ligand of a C-terminal isoleucine or carboxamidato ligand of asparagine. The *cis* configurations appear to be important for the formation of intramolecular hydrogen bonds which stabilize the (hydroxo)iron core structure of ferric SLO-1.

Experimental Section

$Fe(ClO_4)_3 \cdot x H_2O$ and PhCOONa were purchased from Wako Pure Chemical Industries, Ltd. and used without further purification. **Caution**! The perchlorate salts in this study are all potentially explosive and should be handled with care.

tnpa: A solution of tris(6-pivalamide-2-pyridylmethyl)amine[14] (588 mg, 1 mmol) in tetrahydrofuran (20 mL) was added to a mixture of pyridine (20 mL) and $LiAlH_4$ (380 mg, 10 mmol) in tetrahydrofuran (70 mL). The reaction mixture was stirred for 10 h at 70 °C. After cooling, the reaction was stopped by the addition of H_2O. The aqueous mixture was extracted with ethyl acetate. The resulting solution was washed with an aqueous solution of sodium hydrogen carbonate and dried over $MgSO_4$. After removal of the solvent, the product was purified by column chromatography on silica gel with chloroform/methanol as eluent. Needle-like shaped crystals of tnpa were obtained by recrystallization from acetonitrile/diethyl ether (yield: 20%). ^1H NMR (270 MHz, $CDCl_3$, 23 °C, TMS): $\delta = 0.97$ (s, 27H; CH_3), 3.01 (d, $^3J(H,H) = 6.2$ Hz, 6H; $CH_2(CH_3)_3$), 3.68 (s, 6H; NCH_2), 4.52 (t, $^3J(H,H) = 6.2$ Hz, 3H; NH), 6.23 (d, $^3J(H5,H4) = 7.8$ Hz,

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3H; H5), 6.96 (d, ^3J(H4,H3) = 7.8 Hz, 3H; H3), 7.39 (dd, ^3J(H3,H4) = 7.8 Hz, ^3J(H4,H5) = 7.8 Hz, 3H; H4). Elemental analysis (%) calcd for $C_{33}H_{51}N_7$: C 72.62, H 9.42, N 17.96; found: C 72.59, H 9.20, N 17.80.

1: A solution of PhCOONa (72 mg, 0.05 mmol) in H_2O (2.5 mL) was added to a solution of $Fe(ClO_4)_3 \cdot x\,H_2O$ (177.1 mg, 0.5 mmol) and tnpa (273.0 mg, 0.5 mmol) in acetonitrile (10 mL). After stirring for 1 h, the solution was concentrated to give a purple powder of **1**, which was collected by filtration, washed with a small amount of diethyl ether, and dried in vacuo (yield: 89 %). Elemental analysis (%) calcd for $C_{40}H_{59}N_7O_8Cl_1Fe_1$: C 55.70, H 7.15, N 11.37; found: C 56.04, H 6.94, N 11.44.

2: $Fe(ClO_4)_3 \cdot x\,H_2O$ (177.1 mg, 0.5 mmol) was dissolved in methanol containing molecular sieves (3 Å). After 24 h, tnpa (273.0 mg, 0.5 mmol) was added to this solution. After stirring for 30 min, the mixture was concentrated to give a yellow-orange powder of **2**, which was collected by filtration and dried in vacuo (yield: 65 %). Elemental analysis (%)calcd for $C_{35}H_{57}N_7O_6Cl_1Fe_1$: C 55.08, H 7.53, N 12.85; found: C, 54.90, H, 7.72, N, 12.72.

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Keywords: bioinorganic chemistry · enzyme mimetics · iron

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[7] IR spectra of the compounds in acetonitrile at ambient temperature were measured on a ReactIR 1000 spectrometer (ASI Applied Systems) over the range 4000 – 650 cm^{-1}.

[8] Purple crystals suitable for X-ray structure analysis were obtained from an acetonitrile/diethyl ether solution. Crystal data for **1**: $C_{40}H_{59}FeClO_8N_7$, $M_w = 857.25$, monoclinic, space group $P2_1/c$ (No. 14), $a = 10.720(1)$, $b = 16.390(2)$, $c = 26.23(1)$ Å, $\beta = 100.40(2)^\circ$, $V = 4532(1)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.256$ g cm^{-3}, $\mu(Mo_{K\alpha}) = 4.46$ cm^{-1}, $R = 0.079$, and $R_w = 0.113$. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-101084. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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[11] An API 300 triple quadrupole mass spectrometer (PE-Sciex) in positive-ion detection mode, equipped with an ion spray interface, was used for these ESI-MS experiments. The sprayer was held at a potential of 4.5 kV and compressed N_2 was employed to assist liquid nebulization. The orifice potential was maintained at 25 V.

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[13] Another example of a (methoxo)iron(III) complex with such a color (red-orange) is $[Fe\{2,6\text{-}[(2\text{-}C_5H_4N)CH_2OCH_2]_2C_5H_3N\}(OMe)](OSO_2CF_3)_2$ (2-C_5H_4N = 2-Pyridyl, C_5H_3N = 2,6-Pyridindiyl).[6b].

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A Photochemical Switch for Controlling Protein – Protein Interactions**

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Photocaged small molecules and proteins, molecules that can rapidly be converted from inactive into active form with light, have proven to be very useful tools in biology.[1] The ability to control protein – protein binding interactions would further extend this approach to a large number of cellular processes including signal transduction pathways, gene regulation, and protein trafficking. Chemical modification has been used to introduce photocleavable groups into proteins, but this method depends on a uniquely reactive residue being present on the protein surface in order to achieve high selectivity.[2] Here we use unnatural amino acid mutagenesis to photocage the interaction of the $p21^{ras}$ (ras) protein with its downstream effector p120 – GAP (GAP = GTPase-activating protein). The caged ras protein, in which Asp 38 is substituted with the *β-o*-nitrobenzyl ester of aspartic acid (Nb-Asp), retains its intrinsic GTPase activity but is unable to interact

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