One metal-two pathways to the carboxylate-enhanced, iron-containing quercetinase mimics[†]

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Received (in Cambridge, UK) 16th February 2009, Accepted 17th April 2009 First published as an Advance Article on the web 12th May 2009 DOI: 10.1039/b903224j

Mononuclear iron(III) flavonolate was synthesized as synthetic enzyme-substrate complex, and its direct and carboxylateenhanced dioxygenation as biomimetic functional models with relevance to flavonol 2,4-dioxygenase are briefly described.

Flavonol 2,4-dioxygenases (FDO) are enzymes that catalyze the oxidative cleavage of the *O*-heterocycle of polyphenolic flavonols (flaH), which represent a major class of flavonoids.¹ These compounds are important dietary components and have attracted considerable attention owing to their antioxidizing properties.² In the enzymatic process, two C–C bonds are broken, producing more easily degradable depsides (phenolic carboxylic acid esters) and concomitantly carbon monoxide is released (eqn (1)).



FDO enzymes from various species of Aspergillus, Aspergillus flavus,³ Aspergillus niger,⁴ and Aspergillus japonicus⁵ have been characterized, and were found to contain a type II copper ion at the active site. The crystal structure of quercetinase from Aspergillus japonicus shows that the copper center has two alternative conformations, the main form (\sim 70%) being pseudotetrahedral (three histidine residues and a water molecule), and the minor form ($\sim 30\%$) having a mixed trigonal bipyramidal/square pyramidal geometry (side chain of glutamate additionally coordinates the metal).⁵ Interestingly, an X-ray structure of Aspergillus japonicus anaerobically complexed with the natural substrate quercetin indicated that flavonols bind to the copper ion in a monodentate fashion through their O3 atom.⁶ In this geometry, the carbonyl O4 of the flavonol molecule cannot approach the metal, so the substrate chelation (bidentate coordination), generally observed in flavonolato complexes, doesn't occur. А BLAST search conducted against the sequence of

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Aspergillus japonicus identified the YxaG protein from Bacillus subtilis, as the protein with the highest degree of similarity.⁷ Recent studies has been described the YxaG as an iron-containing flavonol dioxygenase with three histidines, one glutamate and one water around the metal center, and their arrangement is similar to that of the Aspergillus japonicus dioxygenase.⁸ It was also found that the packing of the modeled quercetin in the Yxag structure appears to be less tight than in the Aspergillus japonicus.

The synthesis of low molecular weight metal complexes mimicking dioxygenase activity has been challenging for bioinorganic chemists and for many years efforts have been made to obtain catalytically active compounds. Autoxidations of potassium, zinc, iron, manganese and copper flavonolates, mainly at elevated temperatures, have resulted in enzyme-like products, and efforts have also been made to elucidate the mechanisms of these reactions.⁹

The stability of the metal flavonolates above can be explained by the chelation and formation of a stable five-membered ring in the flavonolate complexes. It can be assumed that the coordination mode of the substrate in the enzymatic and model systems could give rise to differences in the degradation rates. In this paper, we report the synthesis and characterization of a Fe^{III}(fla)(salen) [salenH₂ = 1,6-bis-(2-hydroxyphenyl)-2,5-diaza-hexa-1,5-diene] complex as a synthetic model for the iron-containing Yxag dioxygenase, and its direct and carboxylate-enhanced dioxygenation, respectively. We will show that bulky carboxylates as coligands dramatically enhance the reaction rate, which can be explained by two different mechanisms, caused by the formation of more reactive monodentate iron(III) flavonolate complexes.

Complex Fe^{III}(fla)(salen) was synthesized by the reaction between Fe^{III}(salen)(Cl) and flavonol in the presence of triethylamine at room temperature in MeOH. The crystal structure of Fe^{III}(fla)(salen), shown in Fig. 1[±] together with selected data, shows a distorted octahedral geometry around the iron(III) center. and that the flavonolate anion is coordinated as a bidentate ligand with a strongly twisted conformation of the salen ligand. The ⁵⁷Mössbauer spectrum of the complex exhibits a dominant doublet with isomer shift, $\delta = 0.49$ mm s⁻¹ and quadrupole splitting, $\Delta E_Q = 1.44$ mm s⁻¹, indicating a high spin Fe(III) compound.¹⁰ This is consistent with the structure of the complex where iron is surrounded by ligands resulting a considerable asymmetric charge distribution reflected by the obtained quadrupole splitting value. Fe^{III}(fla)(salen) shows absorption in the visible region at 407 nm due to the flavonolate ligand.¹¹ Strong IR band at 1549 cm⁻¹ assigned to ν (CO), showing a decrease of 53 cm⁻¹ compared to flavonol [ν (CO) = 1602 cm^{-1} , arise as a results of the formation of a five-membered ring.12

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[†] Electronic supplementary information (ESI) available: Kinetic measurements data, mass and Mössbauer spectra for Fe(fla)(salen). CCDC 719842. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b903224j



Fig. 1 The molecular structure of $Fe^{III}(fla)(salen)$ with selected bond distances (Å) and angles (°): Fe2–O1 1.899(4), Fe2–O1a 1.935(4), Fe2–O3 1.955(4), Fe2–O2 2.139(4), Fe2–N1 2.141(5), Fe2–N1a 2.080(5), O2–C9 1.272(7), O3–C10 1.318(7), C9–C10 1.432(8), C9–C17 1.414(8), C10–C11 1.363(8), C11–O4 1.370(7), C12–O4 1.354(7), N1–C1 1.462(8), N1–C2 1.273(8), N1a–C1a 1.283(8), N1a–C2a 1.283(8), O1a–Fe2–O2 161.72(16), O3–Fe2–N1 158.85(19), O1–Fe2–N1a 158.29(19). Ellipsoids are shown at the 50% probability level.

Complex Fe^{III}(fla)(salen) is inert to dioxygen in its solid form, and even in solution at ambient conditions. At an elevated temperature (100-120 °C) the dioxygenation reaction proceeds reasonably fast in DMF. The CO content was determined by GC-MS. The GLC-MS analysis of the residue of the hydrolyzed complex, after treatment with ethereal diazomethane, showed the presence of the O-benzoylsalicylic acid methylester. Kinetic experiments were performed in DMF solutions at 90-105 °C. The dioxygenation reactions, followed by measuring the absorbance decrease of the π - π * transition at 407 nm arising from the coordinated flavonolate ligand, afforded a time profile indicating an exponential decay of Fe^{III}(fla)(salen). A straight line was obtained in a plot of the reaction rate versus the initial concentration of Fe^{III}(fla)(salen) and dioxygen to establish a rate law of $-d[Fe^{III}(fla)(salen)]/dt = k[Fe^{III}(fla)(salen)][O_2]$ with kinetic parameters: $k = (2.07 \pm 0.12) \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}, \Delta H^{\#} =$ 76 kJ mol⁻¹, $\Delta S^{\#} = -94$ J mol⁻¹ K⁻¹ at 373.16 K (Table 1).

The influence of the 4'-substituted groups on the reaction rate showed a linear Hammett plot with a reaction constant of $\rho = -0.54$, indicating that the electron-releasing groups result in remarkable increase (*ca.* 2.5-fold) in the reaction rates (Fig. 2).

Besides the electronic factors, the steric effect was also investigated on the dioxygenation reaction. We have found that the rate of dioxygenolysis is dramatically enhanced by



Fig. 2 (a) Steric effects on the reaction rate for the dioxygenation of $Fe^{III}(fla)(salen)$ in the presence of 10 equiv. acetates in DMF at 100 °C (correlation between the number of phenyl substituents of acetates and the relative rates) (b) Substituent effects on the rate constants for the dioxygenation of $Fe^{III}(4'R'-fla)(salen)$ in DMF at 100 °C.

various coligands, such as acetate (CH₃CO₂⁻), phenyl (PhCH₂CO₂⁻), diphenyl (Ph₂CHCO₂⁻) or triphenyl acetate (Ph₃CCO₂⁻) (Fig. 2). For example, addition of 10 equiv. of the bulky Ph₃CCO₂⁻ to Fe^{III}(fla)(salen) accelerated its decay by two order of magnitude ($V_r = 171$) at 100 °C, and the reaction above can take place even at ambient temperatures (20 °C).

The GLC-MS analysis of the hydrolyzed complexes, after treatment with ethereal diazomethane, showed the presence of the O-benzoylsalicylic acid methylester. Provided ^{18,18}O₂ and $^{16,16}O_2$ is used in the dioxygenation, the labeling of the product permits identification of the place of the dioxygen incorporation. As a results of dioxygenations, carried out under an atmosphere containing $\sim 40\%$ ^{18,18}O₂, the ¹⁸O-benzoylsalicylic acid derivative gave a molecular ion at m/z 260 (256 + 4), showing that both ¹⁸O atoms of ^{18,18}O₂ are incorporated into the carboxylic acid from molecular oxygen and the gas phase showed only the presence of unlabeled CO. The kinetics of the carboxylate-enhanced dioxygenation of Fe^{III}(fla)(salen) measured at 40 °C (Fig. 3) resulted in a rate equation with first order dependence on Fe^{III}(fla)(salen), dioxygen and triphenyl acetate ($k' = (5.02 \pm 0.35) \times 10^2 \text{ M}^{-2} \text{ s}^{-1}$, $\Delta H^{\#} = 35 \text{ kJ mol}^{-1}, \Delta S^{\#} = -120 \text{ J mol}^{-1} \text{ K}^{-1} \text{ at } 313.16 \text{ K}$). The main mechanistic difference between the direct and carboxylateenhanced dioxygenation of Fe^{III}(fla)(salen) is that in the latter case there is an electron transfer from Fe^{III}(fla)(salen) to dioxygen resulting in the formation of free $O_2^{\bullet-}$ (ca. 25%), which was proved by the test for free $O_2^{\bullet-}$ with nitroblue tetrazolium (NBT),

Table 1 Kinetic data for the dioxygenation of Fe^{III}(fla)(salen)

	$V_{ m r}^{a}$	$\Delta H^{\#}/\mathrm{kJ}~\mathrm{mol}^{-1}$	$\Delta S^{\#}/J \text{ mol}^{-1} \text{ K}^{-1}$	ρ
Fe(4'R'-fla)(salen)(L)	_	_		_
R' = H; L = -;	1.00	76	-94	-0.54
R' = Cl; L = -;	0.61		_	_
$\mathbf{R}' = \mathbf{OMe}; \mathbf{L} = -;$	1.40			_
$R' = NMe_2; L = -;$	2.45		_	_
$\mathbf{R}' = \mathbf{H}; \mathbf{L} = \mathbf{C}\mathbf{H}_{3}\mathbf{C}\mathbf{O}_{2}^{-};$	19		_	_
$\mathbf{R}' = \mathbf{H}; \mathbf{L} = \mathbf{PhCH}_2\mathbf{CO}_2^-;$	30		_	_
$\mathbf{R}' = \mathbf{H}; \mathbf{L} = \mathbf{Ph}_2 \mathbf{CHCO}_2^{-};$	86		_	_
$\mathbf{R}' = \mathbf{H}; \mathbf{L} = \mathbf{Ph_3CCO_2^{-}};$	171		_	_
$\mathbf{R'} = \mathbf{H}; \mathbf{L} = \mathbf{Ph_3CCO_2^-};$	—	35	-120^{b}	-0.78^{b}
^a In DMF at 100 °C. ^b At 40 °C.				



Fig. 3 (a) (A) Visible spectral change for the decay of $Fe^{III}(salen)(fla)$ in the presence of 10 equiv. $Ph_3CCO_2^-$ in DMF at 40 °C, (B) in the presence of NBT. (b) Time-dependent conversion of $Fe^{III}(fla)(salen)$ under the condition described above, monitored at 407 nm.

where the reduction of the added dye to the blue diformazan took place (Fig. 3).



On the basis of chemical, spectroscopic and kinetic data it can be said that bulky carboxylates as coligands dramatically enhance the reaction rate, which can be explained by two different pathways, caused by the formation of more reactive monodentate flavonolatoiron complexes (eqn (2)). An analogous reaction pathway, *i.e.*, direct electron–electron transfer from the activated flavonol to dioxygen without the need for redox cycling of the metal (b), was suggested for the Ni- and Co-containing flavonol 2,4-dioxygenase.¹⁵ In conclusion, we have shown a significant steric effect on the oxidative decay of $\text{Fe}^{\text{III}}(\text{fla})(\text{salen})$ on addition of a bulky carboxylate such as triphenyl acetate, which accelerates the decay of $\text{Fe}^{\text{III}}(\text{fla})(\text{salen})$ by as much as two orders of magnitude. This probably renders a monodentate coordination of the flavonol to the iron, which is less stable than the bidentate coordination and the higher electron density on C2 makes electron transfer from the flavonolate to O₂ easier. On adding bulky carboxylates the reaction proceeds even at room temperature, which is an unprecedented model. These observations lend strong credence to postulated mechanism of copper and iron-containing dioxygenases.

Financial support of the Hungarian National Research Fund (OTKA K67871 and K75783), COST and Budaconsum Ltd is gratefully acknowledged.

Notes and references

[‡] Intensity data were measured on a Rigaku R-Axis Rapid singlecrystal diffractometer, using a graphite-monochromated Mo- K_{α} radiation ($\lambda = 0.71075$ Å) and ϕ scan technique at 293 K. SHELX-97¹³ was used for structure solution, and full matrix least squares refinement on $F^{2,14}$ CCDC 719842 (Fe(fla)(salen)).[†]

Crystal data: Compound Fe(fla)(salen): C₃₁H₂₃FeN₂O₅, $M_w = 559.37$, triclinic, space group *P*1, a = 10.505(9), b = 12.060(11), c = 13.489(12) Å, $\alpha = 63.650(12)$, $\beta = 67.833(17)$, $\gamma = 85.29(2)^{\circ}$, V = 1411(2) Å³, Z = 2, $D_c = 1.317$ g cm⁻³, μ (Mo- K_{α}) = 0.576 cm⁻¹, 12 569 reflections measured, 2536 parameters refined on F^2 using 3583 unique reflections to final indices $R[F^2 > 2\sigma(F^2)] = 0.0773$, wR = 0.1571, $w = 1/[\sigma^2(F_0^2) + (0.0709P)^2 + 2.7386P]$, $P = (F_0^2 + 2F_c^2)/3$.

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