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Biomimetic O_2 Activation

A Trispyrazolylborato Iron Malonato Complex as a Functional Model for the Acetylacetone Dioxygenase**

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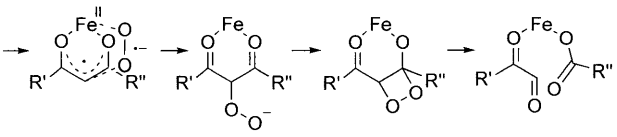
Dedicated to Professor Bernt Krebs on the occasion of his 70th birthday

Acetylacetone dioxygenase (Dke1) is an enzyme that can be isolated from *Acinetobacter johnsonii*, and it catalyses the degradation of acetylacetone, which is toxic to various mammals and to marine creatures and organisms; to achieve this process it consumes one equivalent of dioxygen.[1] Dke1 is also capable of cleaving a whole series of other β diketones and β ketoesters that are derived from acetylacetone by substitution at the 1, 3, or 5 position to yield the corresponding carboxylic acids and α-ketoaldehydes. A 1,3-carbonyl structural motif is decisive for the activity; thus, for example, related 1-keto-3-hydroxo compounds are not converted. Dke1 has been investigated with the aid of single crystal X-ray diffraction and fluorescence and UV/Vis spectroscopy, and from the results obtained its active site is assumed to contain iron(II) coordinated by three histidine residues and water.[2]

The substrate cleavage mechanism is however still largely unsettled. After preliminary studies based on isotopic labeling experiments, an initial deprotonation of acetylacetone followed by attack of dioxygen or superoxide at the C_α postion has been suggested. This mechanism would lead to an organoperoxide unit, which, after nucleophilic attack of its terminal oxygen atom at the carbonyl carbon atom, should decompose via a dioxetane species to give the cleavage products.[3] In the course of further investigations, it was proposed that the role of the metal is restricted to breaking the spin forbiddance in the reaction of triplet dioxygen with the singlet substrate (by interaction of the HOMOs involved), so that the organoperoxo species can be generated in a concerted step (Scheme 1).[4] Thus, a step which plays a decisive role in conversions of many oxygenating heme and non-heme iron enzymes, the binding of dioxygen at the iron(II) center under formation of a Fe^{III}-$O_2^{\bullet}$ entity, has not been considered.



Scheme 1. Proposed mechanism for the cleavage of β-dicarbonyl compounds promoted by acetylacetone dioxygenase.[4]

On the other hand, it has been shown that a substitution of iron(II) by various other metal ions, such as Zn^{2+}, Co^{2+}, Mn^{2+}, Cu^{2+}, or Ni^{2+}, is accompanied by a loss in activity.[2b]

Model compounds can provide valuable support in answering mechanistic questions arising for the reactions of active sites in metalloenzymes.[5] α-Keto acid-dependent non-heme iron enzymes may serve as an example. In these proteins (similarly to the acteylacetone dioxygenase), an Fe^{II} center coordinated by three amino acid residues (two histidines and one asparagine) binds a cofactor through two oxygen donors for a subsequent oxidation with O_2. Model complexes containing the Tp ligand (Tp = hydridotrispyrazol-1-ylborato) have contributed significantly to understanding the protein function. After precoordination of a bidentate substrate analogue to a $\{TpFe^{II}\}$ unit, one coordination site remains available, as is the case in the enzyme, and it was shown that dioxygen is activated at the free site for an attack at the cofactor.[6] Herein we investigate whether a similar scenario is conceivable for acetylacetone dioxygenase with the aid of {TpFe} complexes.[7]

Kitajiama et al. prepared [Tp$^{i\mathrm{Pr}_2}$Fe(acac)] (acac = acetylacetonato, Tp$^{i\mathrm{Pr}_2}$ = hydridotris(3,5-*i*propylpyrazol-1-yl)borato) without any biomimetic motivation. On exposure to air, this compound decomposes within one week to yield a trinuclear iron(III) complex containing μ-oxobis(μ-acetato) and μ-hydroxobis(μ-acetato) ligand constellations between the metal centers.[10] The bridging acetato ligands no doubt have their origin in the acetylacetonato ligands employed, and the question arises as to whether these acetato ligands were generated by a dioxygenating cleavage according to the enzyme reactivity. We therefore synthesized an analogue in which the isopropyl groups at the ligand are replaced by methyl groups to achieve a higher reactivity.[6a] This complex, [Tp*Fe(acac)], Tp* = hydridotris(3,5-dimethylpyrazol-1-yl)borato, was then treated with dry dioxygen. The observed color change from yellow to brown hinted at the formation of iron(III) compounds, and subsequent ESI-MS studies detected the species $[FeTp^*_2]^+$, $[Tp^*Fe(acac)]^+$, and $[(Tp^*Fe)_2O(acac)]^+$, that is, the acetylacetonato ligand had

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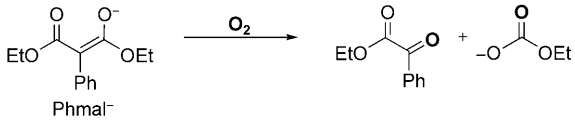
remained intact and cleavage products could not be detected (see the Supporting Information). Iron complexes containing acetato ligands could only be detected when water was added to the reaction mixture, so it can be assumed that the acetato ligands of the previous studies are at least partly due to the water present under aerobic conditions (hydrolysis of the acetylacetonato ligand[11] following oxidation of the complex). However, water is clearly not involved in the cleavage of acetylacetone by Dke1, since in a hydrolytic degradation acetone would accompany acetate as a product and not—as observed—pyruvaldehyde. Thus the system [TpFe(acac)]/O_2/H_2O is not an adequate model.

Accordingly, modeling of the β-diketone cleavage with dioxygen requires a more reactive substrate ligand. Bearing in mind that it is desirable to detect the 1,2-dicarbonyl product which is potentially sensitive towards further oxidation, this ligand should be substituted at least at the C_α position. Considering that β ketoesters are also cleaved by Dke1, we chose diethyl malonate with a phenyl substituent in the C_α position (HPhmal) as a model substrate. This choice was further inspired by our recent observation for a derivative that, in the presence of iron(II) without polydentate co-ligands, the malonate unit undergoes unselective oxidation[8]). The oxidation products expected for cleavage by analogy to Dke1, namely $EtOCO_2^-$ and ethyl benzoylformate, can neither be oxidized any further nor enolized for a subsequent cleavage reaction (Scheme 2).

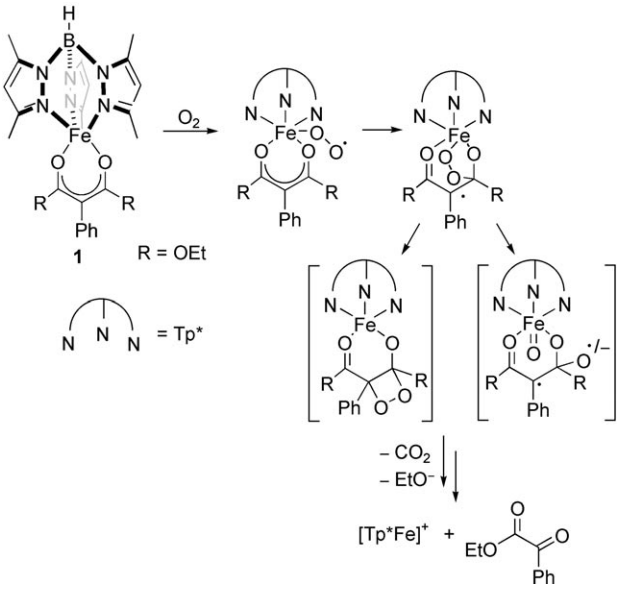


Scheme 2. Expected products resulting from dioxygenation of diethyl phenylmalonate according to the reactivity of Dke1.

The synthesis of complex [Tp*Fe(Phmal)] (**1**; Scheme 3) was thus pursued and finally achieved by treatment of KTp* and LiPhmal dissolved in acetonitrile with $FeCl_2$. After workup, analytically pure **1** was obtained in 51% yield. Compound **1** was crystallized by slow cooling of a saturated solution of **1** in hexane. The result of a single crystal X-ray diffraction study is given in Figure 1.[12]

The iron(II) ion is located in a distorted trigonal bipyramidal coordination sphere ($\tau = 0.58$)[13] that is composed of the three nitrogen atoms of the Tp* ligand and the oxygen atoms of the substrate. As expected, a vacant coordination site is available at the iron(II) center for the complexation and activation of dioxygen.

After treating of an acetonitrile solution of **1** with dry dioxygen at room temperature, workup with 0.5 mL of 3 M HCl, followed by filtration employing a silica gel column and acetonitrile as eluent, led to a solution containing the HPhmal cleavage products. A GC-MS (CI) analysis revealed only one peak ($m/z = 179.1$) which, as confirmed by ^1H and ^{13}C NMR spectroscopy,[14] corresponds to ethyl benzoylformate. As outlined above, ethyl benzoylformate is one of the two products expected for dioxygenase activity of **1** analogous to



Scheme 3. Conceivable mechanism for the oxidative cleavage of diethyl phenylmalonate.

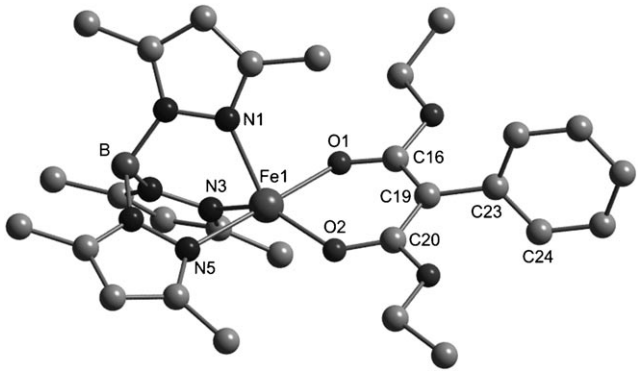


Figure 1. Molecular structure of **1**. All hydrogen atoms were omitted for clarity. Selected bond lengths [Å] and angles [°]: Fe1–O1 = 2.0876(8), Fe1–O2 = 1.9868(9), Fe1–N1 = 2.0968(11), Fe1–N3 = 2.0906(11), Fe1–N5 = 2.1678(10), O2–C20 = 1.2695(14), O1–C16 = 1.2444(15), C16–C19 = 1.4188(17), C19–C20 = 1.4026(16); C16-C19-C20 = 118.74(11), O2-Fe1-N3 = 142.34(4), O1-Fe1-N5 = 177.13(4), C16-C19-C23-C24 = 71.99(16).

the function of Dke1 (see Scheme 2). The second product, $EtOCO_2^-$, appears to decompose during the reaction to give ethanolate and carbon dioxide; the latter could be detected by IR spectroscopy. To obtain further evidence for dioxygenase reactivity, additional experiments were carried out in which compound **1** was treated with ^{18}O-enriched dioxygen (95%). Subsequent GC-MS analysis revealed the incorporation of one ^{18}O atom into the α keto ester with an efficiency of 94%.[15] Incorporation of the second oxygen atom into carbon dioxide was confirmed by IR spectroscopy; the ν_{as} absorption band shifted by about 16 cm^{-1}, as expected for $^{18}OC^{16}O$.[16]

The reaction was also monitored by ^1H NMR spectroscopy. If a solution of **1** in CD_3CN is exposed to dioxygen, the paramagnetically shifted signals of **1** disappear within 2 h and signals corresponding to ethyl benzoylformate and two sets of

signals for ethanolate species appear in their place. Further signals could not be detected, so the fate of the {Tp*Fe} fragment was initially unclear. However, within a few hours, crystals of [Tp*$_2$Fe] precipitated from the solution. The formation of this species requires the generation of a Tp*-free iron compound, which then carries the ethanolate ligands.

Next, the question of whether malonate cleavage requires the activation of dioxygen at the iron(II) center was addressed. LiPhmal was reacted with dioxygen. After workup in an analogous manner to the reaction of **1** with dioxygen, products from cleavage could not be identified, so that the C=C cleavage appears to require more than just a Lewis acidic metal center. Moreover, **1** was oxidized with $NOPF_6$, and the resulting iron(III) compound was treated with dioxygen. Again, the usual workup did not lead to the detection of cleavage products by GC/MS analysis, so we assume that, in contrast to the mechanism proposed for the enzyme, the reaction of **1** takes place by activation of dioxygen at the iron(II) center to give an iron(III)–superoxido species. The terminal oxygen atom then attacks at the electrophilic carbonyl carbon atom of the Phmal ligand, leading to an iron-organoperoxido unit (Scheme 3), as similarly suggested for model complexes for α-keto acid-dependent iron enzymes.[6] Subsequently, either a dioxetane species is formed, decomposing by cycloreversion to give the cleavage products, or O–O bond cleavage (homolytic or heterolytic) occurs. The latter would have to proceed with the concomitant formation of a high-valent iron oxido species, which should oxygenate the C_α atom. Attack of the terminal oxygen atom of the iron superoxido species directly at the nucleophilic C_α atom seems unlikely. The fact that, after oxidation with $NOPF_6$ and subsequent reaction with dioxygen, cleavage was not observed also excludes a mechanism involving intramolecular electron transfer and formation of a substrate-centered radical by analogy to the intradiol-cleaving catechol dioxygenases.[6c–f]

Scheme 3 clearly shows that all four oxidation equivalents of dioxygen are compensated exclusively by the ligand, such that after the cleavage, the iron center has the oxidation state +II again. Consequently, catalytic conversions should be possible, and to test this hypothesis a mixture of PhmalLi and **1** (5 mol%) was treated with dioxygen. Workup and analysis revealed selective conversion into ethyl benzoylformate, carbon dioxide, and ethoxide as before; now, however, the reaction occurs catalytically with a TOF of 55 h^{-1}.

Thus, compound **1** is an excellent model for the active site of acetylacetone dioxygenase, as it meets three criteria: 1) structural similarity: the Tp* ligand mimics the $(His)_3$ coordination sphere of the iron(II) center, and diethyl phenylmalonate is a suitable substrate analogue for β diketonates and β ketoesterates; 2) the function is also simulated: upon contact with dioxygen, ethyl benzoylformate and carbon dioxide are selectively formed, and the dioxygenase activity was confirmed by $^{18}O_2$ experiments; and 3) as in the case of the enzyme, the model also acts catalytically.

Consequently, the dioxygen activation mechanism deduced for the model compound **1** is an interesting new hypothesis for the enzyme function, especially as it would also be in accordance with the results of kinetic studies performed with the enzyme.[4]

Experimental Section

All manipulations were carried out in a glove box, or by Schlenk techniques involving the use of a dry argon atmosphere. Solvents were purified, dried, and degassed prior to use. Me_2PzH,[17] KTp*,[18] and lithium diisopropylamide[19] (LDA) were prepared according to literature procedures. ^{18}O-enriched dioxygen (95%) was purchased from Chemotrade. The lithium salt of diethyl phenylmalonate was prepared by stirring 1 equivalent of LDA with diethyl phenylmalonate in THF.

1: $FeCl_2$ (376 mg, 2.97 mmol) was added to a solution of lithium diethyl phenylmalonate (720 mg, 2.97 mmol) and of KTp* (1.00 g) in acetonitrile (50 mL). The reaction mixture was stirred overnight and a small quantity of [Tp*$_2$Fe] was then filtered off. The filtrate was evaporated to dryness, and the resulting white residue was washed twice with hexane (20 mL). Thereafter **1** was extracted three times with hexane (60 mL), and all volatiles were removed from the combined solutions in vacuo. The resulting white powder was washed twice with hexane (15 mL) to give analytically pure **1** (904 mg, 51% yield, 1.54 mmol). Crystals of **1** suitable for single crystal X-ray diffraction studies were obtained by cooling a saturated solution of **1** in hexane. ^{1}H NMR (CD_3CN, 25°C) $\delta = -13.66$ (4H, CH_2), −2.03 (6H, CH_3), 5.93 (1H, CH_{Ar}), 6.03 (9H, Pz-CH_3), 6.96 (2H, CH_{Ar}), 8.46 (2H, CH_{Ar}), 12.25 (9H, Pz-CH_3), 56.59 ppm (3H, 4H-Pz). IR: $\tilde{\nu} = 2984$ (w), 2928 (w), 2523 (s), 1624 (vs), 1599 (m), 1543 (m), 1450 (s), 1410 (s), 1379 (s), 1333 (s), 1313 (s), 1271 (vw), 1198 (m), 1011 (w), 806 (m), 789 cm^{-1} (m). Elemental analysis (%) calcd for $C_{28}H_{37}BFeN_6O_4$ (588.23 g mol^{-1}): C 57.17, H 6.34, N 14.29; found: C 56.72, H 6.25, N 14.20. UV/Vis (MeCN) $\lambda = 260$ nm; $\chi_m = 1.05 \times 10^{-2}$ $cm^3 mol^{-1}$, $\mu_{eff} = 5.00$ μ_B ($\mu_{so} = 4.90$ μ_B).

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α keto acid–iron(II) compound with dioxygen led to partial C–C bond cleavage adjacent to the carbonyl group.[9]

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[12] X-ray structure analysis of **1**: $C_{28}H_{37}BFeN_6O_4$, $M_r = 588.30$, monoclinic, $P2_1/c$, $a = 9.8480(4)$, $b = 19.0788(8)$, $c = 16.2832(6)$ Å, $\beta = 101.146(3)°$, $V = 3001.7(2)$ Å^3, $Z = 4$, $T = 100(2)$ K, $F_{000} = 1240$, $\mu = 0.545$ mm^{-1}, $\Theta = 2.55$–$29.00°$, reflections collected 30965, independent reflections 7909 ($R_{int} = 0.0373$), GoF = 0.992, $R_1 = 0.0297$, $wR_2 = 0.0709$, largest diffraction peak/hole 0.348/−0.300 e Å^{-3}. The crystal was mounted on a glass fiber and then transferred into the cold nitrogen gas stream of the diffractometer (Stoe IPDS2N). $Mo_{K\alpha}$ radiation, $\lambda = 0.71073$ Å. The structure was solved by direct methods (SHELXS-97),[20] refined versus F^2 (SHELXL-97)[21] with anisotropic temperature factors for all non-hydrogen atoms. All hydrogen atoms were added geometrically and refined by using a riding model. CCDC-692202 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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