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Bioinspired Oxidative Cleavage of Aliphatic C-C Bond Utilizing Aerial Oxygen by Nickel Acireductone Dioxygenase Mimics

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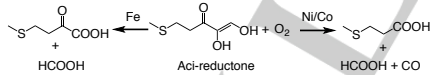
Abstract: Bioinspired oxidative cleavage of aliphatic C-C bonds of acireductone model substrate, 2-hydroxy-3-oxo-1,3-diphenylprop-1-en-1-olate, bound to two paramagnetic nickel(II) complexes that are mimics for the nickel containing acireductone dioxygenase has been achieved utilizing aerial oxygen under ambient conditions.

Introduction

Nickel-containing enzymes assist the catalysis of many important biological processes. Most of them are significant in the context of industry and environment, such as **(1)** hydrolysis of urea to ammonia (urease),[1-4] **(2)** interconversion of carbon monoxide and carbon dioxide (carbon monoxide dehydrogenase), **(3)** acetyl group metabolism into separate one-carbon units or acetate synthesis using one-carbon precursors (acetyl-coenzyme A synthase),[5-13] **(4)** isomerization of hemithioacetal in order to detoxify the cytotoxic methylglyoxal (glyoxalase I),[14-16] **(5)** reversible interconversion of dihydrogen into protons and electrons (NiFe hydrogenase),[17-21] **(6)** degradation of methylenediurea, a slow release fertilizer (methylenediurease),[22] **(7)** methane generation (methyl-coenzyme M reductase),[23] **(8)** dismutation of toxic and cell damaging superoxide radical anions into harmless molecular oxygen (nickel superoxide dismutase)[24,25] and on top of all, **(9)** oxidation of 1,2-dihydroxy-3-keto-5-methylthiopentene (aci-reductone) into methylthio propionic acid, formic acid and carbon monoxide (acireductone dioxygenase, ARD; Scheme 1).[26-30]



Scheme 1. Metal dependent reactions catalyzed by ARD.

The ARD is known since 1993 as part of the study of the methionine salvage pathway in *Klebsiella pneumoniae*;[31] it was found to cleave the key intermediate of this pathway, aci-reductone and its analogues.[32,33] Investigations using *K. pneumoniae* revealed that acireductone is oxidized to two different sets of products. A dioxygenase activity produces the α-ketoacid precursor of methionine and formic acid in the productive case;[29,32] in a second, non-productive case, the aci-reductone is converted into formate, carbon monoxide, and methylthiopropionic acid. Curiously, both activities stem from the same protein (ARD), but result from the differences in metal content. Further investigations using recombinant protein confirmed that iron-containing ARD (Fe-ARD) is responsible for the productive activity and the non-productive activity is from the Ni– or Co–containing ARD (Ni-ARD or Co-ARD).[32] The overall structure of ARD was elucidated employing high-resolution NMR spectroscopy,[28,30] while the active site structure was studied using X-ray crystallography in recent years.[34] The active site found in mammalian ARD appears to possess an octahedral geometry with three oxygen donors provided by Glu94 and two water molecules in addition to three nitrogen donors provided by His88, His90 and His133 (Fig. 1); each histidine is trans located to an oxygen donor at the paramagnetic nickel(II) ion.[34]

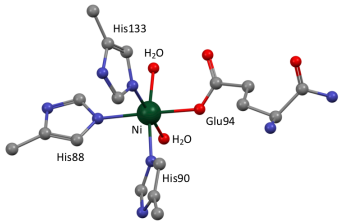


Figure 1. Solid-state structure of the active site of ARD found in *Mus musculus* (Ni-MmARD) with Ni(II) ion (extracted from pdb 5I91).[34]

A limited number of structural and functional models have been reported since 2004 in an effort to understand the catalytic mechanism of ARD by Berreau[35-44] and Mayilmurugan.[45] Berreau employed the paramagnetic Ni(II) complexes of 6-Ph_2TPA[35-43] and 6-PhTPA[44] as mimics of Ni-ARD while Mayilmurugan used $[Ni(BBP)(H_2O)Cl_2]$[45] as the starting point.

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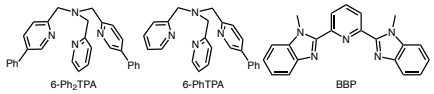


Figure 2. The ligands used by Berreau (6-Ph_2TPA, 6-PhTPA)[35-44] and Mayilmurugan (BBP)[45] to mimic the active site of Ni-ARD.

The first report of Berreau described the aliphatic carbon-carbon bond cleavage reactivity of a Ni(II) *cis*-β-keto-enolate complex, $[(6\text{-}Ph_2TPA)Ni(A2)]ClO_4$ (Fig. 2, 3) in the presence of two equivalents of base and oxygen; $[(6\text{-}Ph_2TPA)Ni(A2)]^+$ reacted with O_2 to produce $[(6\text{-}Ph_2TPA)Ni(OOCPh)_2(H_2O)]$ and CO.[35] Though the model substrate A2 that is closely relevant to aci-reductone underwent C-C cleavage, substrates such as A1, A3 and A4 were unreactive towards dioxygen; the complexes $[(6\text{-}Ph_2TPA)Ni(A3)]ClO_4$ and $[(6\text{-}Ph2TPA)Ni(A1)]ClO_4$ were found to be unreactive towards dioxygen (Fig. 2, 3).[36] In contradiction, $[Ni(BBP)(A3)(H_2O)Cl_2]$ is shown to be reactive towards dioxygen;[45] BBP is a linear tridentate ligand while all the models reported by Berreau possessed tris-picolylamine based tripodal tetradentate ligands such as 6-Ph_2TPA[35-43] and 6-PhTPA[44] (Fig. 2).

In our view, Mayilmurugan's model is closer to the resting state of Ni-ARD as it possesses N_3O_3 donor set while all the successful functional mimics of Berreau have N_4O_2 donor sets. Hence one might wonder if subtle changes in the N4 ligand system would still yield working models or not. To test this, we have chosen the macrocyclic N4 tetradentate ligands L1 and L2 (Fig. 3).

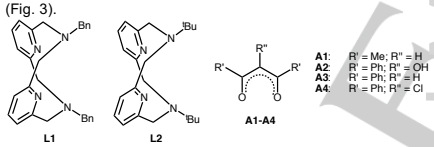


Figure 3. Pyridinophane macrocyclic ligands (L1 and L2) and aci-reductone model substrates (A1-A4) chosen for the present study.

Here we report two new Ni(II) complexes of macrocyclic N4 tetradentate ligands, their adducts with substrates A1-A4 and the reactivity of the adducts towards aerial oxygen (Fig. 3). The complexes and adducts are characterized using NMR spectroscopy, ESI-Mass spectrometry and X-ray crystallographic techniques appropriately.

Results and Discussion

The straight forward reactions of $NiCl_2{\cdot}6H_2O$ and L1 or L2 yielded $[Ni(L1)Cl_2]$ and $[Ni(L2)Cl_2]$ in good yields. Preliminary analysis using high resolution electrospray ionization mass spectrometry (ESI-MS) confirmed the formation of $[Ni(L1)Cl_2]$ (calcd. *m/z* for $[Ni(L1)Cl]^+$ is 513.1356 and observed *m/z* is 513.1355; Fig. S3, S4) and $[Ni(L2)Cl_2]$ (calcd. *m/z* for $[Ni(L2)Cl]^+$ is 445.1669 and observed *m/z* is 445.1642; Fig. S5, S6). Furthermore, solid-state structure of $[Ni(L1)Cl_2]$ exhibited a distorted octahedral geometry around Ni(II) ion (Fig. 4); the distortion is enforced by the tetradentate N4 ligand that folded to coordinate two basal and two apical coordination sites leaving two adjacent positions for labile ligands.

The green suspensions of $[Ni(L1)Cl_2]$ or $[Ni(L2)Cl_2]$ in acetonitrile was mixed and stirred with 2.2 equivalents of $NaBPh_4$ to obtain purple color $[Ni(L1)(CH_3CN)_2](BPh_4)_2$ or $[Ni(L2)(CH_3CN)_2](BPh_4)_2$; the ESI-MS envelopes observed at *m/z* 239.0821 and 205.0904 that correspond to $[Ni(L1)]^{2+}$ (calcd. *m/z* = 239.0833) and $[Ni(L2)]^{2+}$ (calcd. *m/z* = 205.0990) confirmed the anion exchange (Fig. S7-S10). The solid-state structure of the purple crystals of the later exhibited the molecular composition as $[Ni(L2)(CH_3CN)_2](BPh_4)_2{\cdot}CH_3CN$, which was used in further studies.

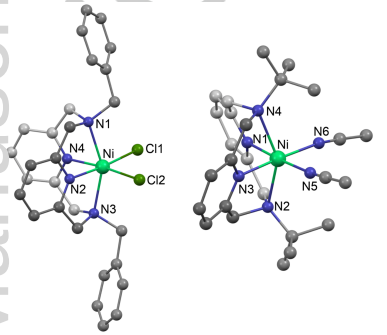
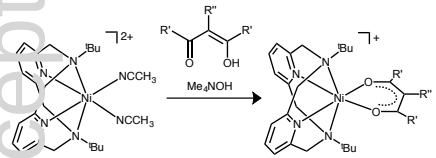


Figure 4. Solid-state structures of $[Ni(L1)Cl_2]{\cdot}H_2O$ (left) and $[Ni(L2)(CH_3CN)_2](BPh_4)_2{\cdot}CH_3CN$ (right). Solvents, counter anions and hydrogens are omitted for sake of clarity. Selected bond distances [Å] of $[Ni(L1)Cl_2]$: Ni-N(2), 2.013(3); Ni-N(4), 2.023(3); Ni-N(1), 2.219(3); Ni-N(3), 2.228(3); Ni-Cl(1), 2.3908(10); Ni-Cl(2), 2.4584(9). Selected bond distances [Å] of $[Ni(L2)(CH_3CN)_2](BPh_4)_2$: Ni-N(3), 1.972(5); Ni-N(1), 1.982(5); Ni-N(6), 2.055(6); Ni-N(5), 2.076(6); Ni-N(2), 2.268(5); Ni-N(4), 2.280(5). More details are provided as supporting information (Figures S1, S2; Tables S1, S2).



Scheme 2. Synthesis of mimics of enzyme-substrate adducts.

Equimolar mixtures of $[Ni(L1)(CH_3CN)_2](BPh_4)_2$ or $[Ni(L2)(CH_3CN)_2](BPh_4)_2$, protonated precursors of adducts (HA1-HA4) and tetramethylammonium hydroxide yielded the mimics of enzyme-substrate adducts $[Ni(L1)(A1\text{-}A4)](BPh_4)$ or $[Ni(L2)(A1\text{-}A4)](BPh_4)$ in good yields. The ESI-MS envelopes indicated the formation of the adducts (Fig. S11-S16); the SCXRD analyses of $[Ni(L2)(A1)](BPh_4)$, $[Ni(L2)(A3)](BPh_4)$ and $[Ni(L2)(A4)](BPh_4)$ exhibited the molecular structure of the adducts (Fig. 5). In all the

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three isolated mimic-substrate adducts the anionic substrate is bound to the nickel center as bidentate chelate as hypothesized earlier.[35]

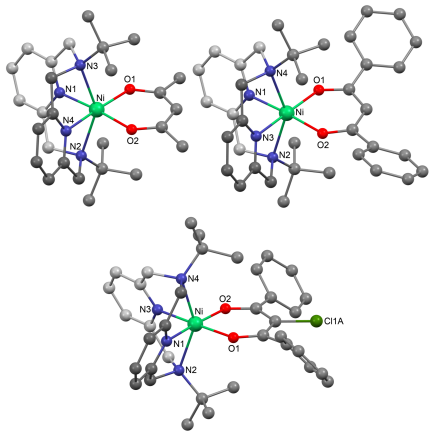
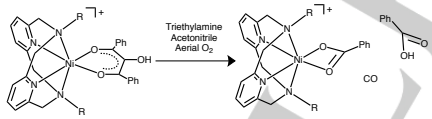


Figure 5. Solid-state structures of $[Ni(L2)(A1)](BPh_4)$ (left), $[Ni(L2)(A3)](BPh_4)$ (right) and $[Ni(L2)(A4)](BPh_4)$ (bottom). Solvents, counter anions and hydrogens are omitted for sake of clarity. Selected bond distances [Å] of $[Ni(L2)(A1)](BPh_4)$: Ni-N(4), 1.9877(18); Ni-O(1), 1.9986(15); Ni-N(1), 2.0000(19); Ni-O(2), 2.0023(15); Ni-N(3), 2.3201(19); Ni-N(2), 2.3252(19). Selected bond distances [Å] of $[Ni(L2)(A3)](BPh_4)$: Ni-O(1), 1.978(4); Ni-N(1), 1.980(5); Ni-N(3), 1.983(5); Ni-O(2), 1.994(4); Ni-N(2), 2.291(4); Ni-N(4), 2.320(4). Selected bond distances [Å] of $[Ni(L2)(A4)](BPh_4)$: Ni-N(1), 1.971(5); Ni-O(2), 1.979(4); Ni-N(3), 1.979(4); Ni-O(1), 1.995(3); Ni-N(2), 2.290(4); Ni-N(4), 2.293(4). More details are provided as supporting information (Figures S17-S19; Table S3-S5).



Scheme 3. Aliphatic C-C bond cleavage of A2 in the presence of triethylamine and aerial oxygen at room temperature (R = Bn or ^{t}Bu).

The oxidative cleavage of model acireductone substrates (A1-A4) have been carried out using a mixture of $[Ni(L1)(CH_3CN)_2](BPh_4)_2$ or $[Ni(L2)(CH_3CN)_2](BPh_4)_2$ and the substrate precursors HA1-HA4 in addition to 4 equivalents of triethylamine in acetonitrile under ambient conditions in open air (Scheme 3). The resulting solutions were analyzed preliminarily employing ESI-MS and it was observed that the substrates A1, A3 and A4 did not undergo C-C bond cleavage instead stable substrate-adduct species were observed (Fig. S20-S22); this is in line with the observations of Berreau.[36] As expected, the substrate A2, that is closer to the acireductone (RC(=O)C(-OH)=CHOH), underwent oxidative C-C bond cleavage utilizing aerial oxygen, as the envelopes for $[Ni(L1)(OOCPh)]^+$ (observed *m*/*z* is 599.1993; calcd. *m*/*z* is 599.1957; Fig. S23, S24) and $[Ni(L2)(OOCPh)]^+$ (observed *m*/*z* is 531.2289; calcd. *m*/*z* is 531.2270; Fig. S25, S26) were present in the ESI-MS (Scheme 3). In addition, the ^{1}H NMR of isolated organic components upon protonation confirms the presence of benzoic acid (Fig. S27).

To the best of our knowledge, the enzyme-substrate models reported to date reactive towards dioxygen only when the substrate is 2-hydroxy-3-oxo-1,3-diphenylprop-1-en-1-olate (A2) that is closer to the natural substrate, acireductone. Only in one case where the oxidative cleavage is initiated photochemically, the substrate A4 is cleaved.[41] Even though, $[Ni(BBP)(A3)(H_2O)Cl_2]$ undergoes C-C cleavage in the presence of dioxygen, the mechanism proposed involves binding of oxygen on Ni whereas the wild-type enzyme-substrate mimic does not go through such intermediate;[45] owing to the labile aqua ligand bound to the $[Ni(BBP)(A3)(H_2O)Cl_2]$, the proposed [Ni-O-O-C-substrate]‡ intermediate might be possible.[45]

Conclusions

In summary, we have shown that two paramagnetic nickel(II) complexes of macrocyclic N4 ligands are able to perform as enzyme-substrate mimic for nickel containing acireductone dioxygenase enzyme. For the first time, aerial oxygen was utilised in the oxidative cleavage of aliphatic C-C bonds of acireductone model substrate. Our future works are dedicated towards design and synthesis of N3O ligands and their Ni(II) complexes with an anionic O donor as such type of models would be closer to the natural ligand environment of acireductone dioxygenase active site.

Supporting Information Summary

The experimental details, ^{1}H NMR and ESI-mass spectra, ORTEPs, crystallographic details are available in the Supporting Information. CCDC 1881017 ($[Ni(L1)Cl_2]$), 1881018 ($[Ni(L2)(CH_3CN)_2](BPh_4)_2{\cdot}CH_3CN$), 1881019 ($[Ni(L2)(A1)](BPh_4)$), 1881020 ($[Ni(L2)(A3)](BPh_4)$) and 1881021 ($[Ni(L2)(A4)](BPh_4)$) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: Acireductone dioxygenase • Enzyme-substrate mimic • Nickel complexes • Aerial oxidation • Aliphatic C-C cleavage

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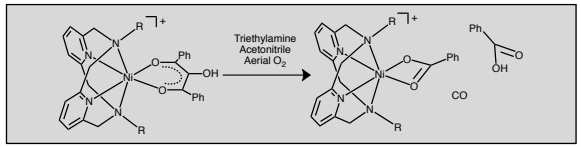

Nickel Aci-reductone Dioxygenase Mimic: Bioinspired oxidative cleavage of aliphatic C-C bonds of acireductone model substrate, 2-hydroxy-3-oxo-1,3-diphenylprop-1-en-1-olate, bound to two paramagnetic nickel(II) complexes that are mimics for the nickel containing acireductone dioxygenase has been achieved utilizing aerial oxygen under ambient conditions.