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Simple Ligand Effects Switch a Hydrogenase Mimic between H_2 and O_2 Activation

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Abstract: Herein, we report a [NiRu] biomimetic system for O_2-tolerant [NiFe]hydrogenases and demonstrate that electron donation to the [NiRu] center can switch the system between the activation of H_2 and O_2 through simple ligand effects by using hexamethylbenzene and pentamethylcyclopentadienyl ligands, respectively. Furthermore, we present the synthesis and direct observations of a [NiRu]–peroxo species, which was formed by the oxygenation of a Ni-SIa model [NiRu] complex, that we propose as a biomimetic analogue of O_2-bound species (OBS) of O_2-tolerant [NiFe]hydrogenases. The [NiRu]–peroxo complex was fully characterized by X-ray analysis, X-ray photoelectron spectroscopy (XPS), mass spectrometry, and ^{1}H NMR spectroscopy. The OBS analogue was capable of oxidizing *p*-hydroquinone and sodium borohydride to turn back into the Ni-SIa model complex.

Keywords: bioinorganic chemistry · biomimetic compounds · hydrogen · ligand effects · oxygen

Introduction

Hydrogenases (H_2ases) are a class of enzymes that are currently receiving much interest owing to their ability to extract electrons, and hence energy, from H_2.[1] Their active centers are based on an [Fe], [FeFe], or [NiFe] core[2–4] and they are usually deactivated by the presence of trace amounts of O_2.[1,5–12]

However, a subset of [NiFe]H_2ases exhibit O_2 tolerance,[7–12] and the two currently favored proposals to explain this O_2 tolerance are physical active site blocking[7–9] and the reduction of bound O_2 by electron-transport from {Fe-S} clusters.[9–12] Evidence for the physical protection of the active site comes from computational modeling and X-ray structures that suggest the presence of small, hydrophobic gas channels in *Ralstonia eutropha* H16;[7] these gas channels would be expected to prevent the diffusion of O_2 to the active site. Support for the electron-transport mechanism has been provided in the form of structural, experimental, and kinetic studies on [NiFe]H_2ases of *Hydrogenovibrio marinus* H110[10] and *Ralstonia eutropha* H16.[11]

It has been proposed that electron transport followed by protonation allows the enzyme to reduce O_2 and eliminate it as water (Figure 1). However, there has been no direct experimental evidence that Ni-SIa (the EPR-silent active state) is able to activate O_2 in this manner and no O_2-bound species (OBS) have been observed so far.

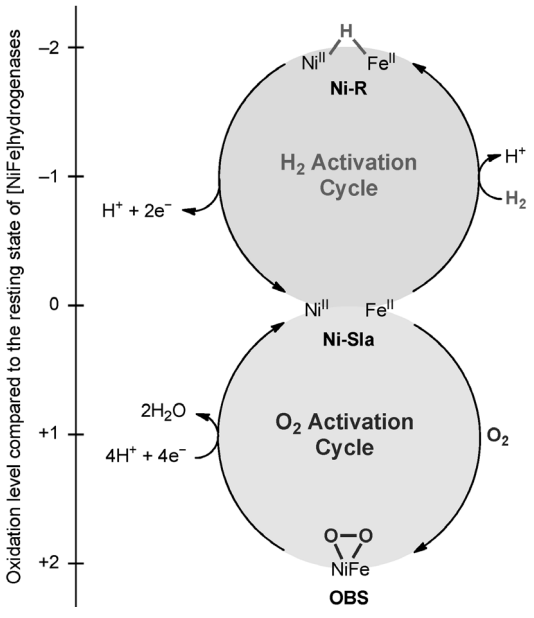

Figure 1. A proposed electron-transport cycle of O_2-tolerant [NiFe]-H_2ases.[11] Ni-SIa: EPR-silent active state. Ni-R: EPR-silent reduced state. OBS: O_2-bound species.

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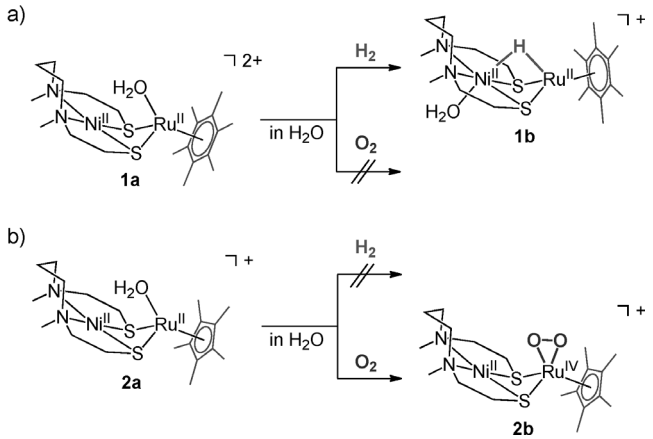
As part of our efforts to apply insights from H_2ases to find solutions for sustainable power, we have previously reported the synthesis of a biomimetic analogue of [NiFe]-H_2ases: $[Ni^{II}LRu^{II}(H_2O)(\eta^6\text{-}C_6Me_6)](NO_3)_2$ ([**1a**]$(NO_3)_2$; L = *N*,*N*′-dimethyl-*N*,*N*′-bis(2-mercaptoethyl)-1,3-propanediamine). This organometallic catalyst was capable of reproducing all of the defining chemical aspects of these enzymes: heterolytic activation of H_2, electron extraction from H_2, and isotope-exchange with D_2O to simultaneously generate HD and D_2.[13] This unique ability to thoroughly mimic the properties of [NiFe]H_2ases gave us confidence in using it as an organometallic substitute for the real enzymes. In addition, because this system was wholly artificial and relatively simple, we were able to make a systematic evaluation of its structural and electronic characteristics.[13]

In common with O_2-tolerant [NiFe]H_2ases, compound **1a** could activate H_2 even in the presence of O_2 (Scheme 1a). Whilst we could not "catch it in the act" of interacting with O_2, we considered that a relatively small change to its electronic properties might enhance any interactions to the point of observability.



Scheme 1. Reactivity of a) compound **1a** and b) compound **2a** towards H_2 and O_2 in water.

With this goal in mind, we synthesized a variation of compound **1a** that replaced the hexamethylbenzene ligand with a pentamethylcyclopentadienyl ligand: $[Ni^{II}LRu^{II}(H_2O)(\eta^5\text{-}C_5Me_5)](NO_3)$, [**2a**]$(NO_3)$. The pentamethylcyclopentadienyl anion is a stronger σ-donor than hexamethylbenzene and thus this substitution allowed us to better establish the importance of aromatic electronic donors to the reactivity of our system, whilst providing an insight into O_2 activation. Electron donation was especially relevant to the study of these H_2ases because it has been suggested that a proximal {Fe-S} cluster can provide electrons to activate bound O_2 at the active site and thereby facilitate its removal.

Herein, we report that σ-donation to the Ru center of compound **2a** switched the activity of the system between H_2 and O_2 activation. We also provide crystallographic evidence for a [NiRu]–peroxo complex, which may act as a biomimetic analogue of the OBS of H_2ases, and thereby shed light on the mechanism of the O_2 tolerance.

Results and Discussion

Two versions of our system were synthesized: compounds **1a** and **2a**. The synthesis of compound **1a** has been reported previously,[13] whilst complex [**2a**](NO_3) was synthesized by the treatment of a Ni^{II} complex, $[Ni^{II}L]$,[14] with Ru^{II} complex $[Ru^{II}(\eta^5\text{-}C_5Me_5)(CH_3CN)_3](NO_3)$[15] in water and was isolated as a black powder. The structure of compound **2a** was characterized by electrospray ionization mass spectrometry (ESI-MS), ^{1}H NMR and IR spectroscopy, and elemental analysis.

We examined the reactivity of compounds **1a** and **2a** toward 0.1 MPa of H_2 or O_2 in water at 25 °C (Scheme 1). H_2 or O_2 gas was bubbled through aqueous solutions of compounds **1a** or **2a**, and the reactions were monitored by ESI-MS and UV/Vis spectroscopy. As previously reported, compound **1a** activated H_2 to form a hydride complex, $[Ni^{II}(H_2O)L(\mu\text{-}H)Ru^{II}(\eta^6\text{-}C_6Me_6)](NO_3)$ ([**1b**](NO_3)),[13] but showed no reactivity toward O_2. By contrast, compound **2a** did not react with H_2 but acquired O_2 to afford a $Ni^{II}Ru^{IV}$–peroxo complex, $[Ni^{II}LRu^{IV}(\eta^2\text{-}O_2)(\eta^5\text{-}C_5Me_5)](NO_3)$ ([**2b**](NO_3)). Compound **2b** was characterized by X-ray analysis (Figure 2), X-ray photoelectron spectroscopy (XPS, Figure 3), ESI-MS (Figure 4), ^{1}H NMR and IR spectroscopy, and elemental analysis.

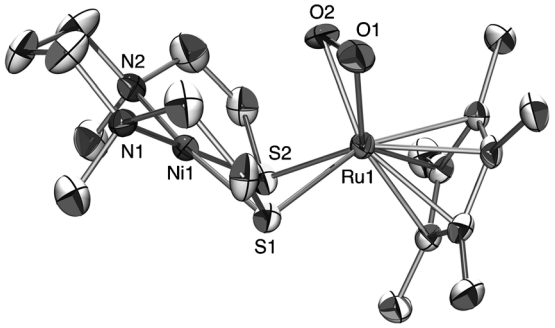


Figure 2. An ORTEP of complex [**2b**](CF_3SO_3), ellipsoids set at 50% probability. The counteranion (CF_3SO_3) and hydrogen atoms are omitted for clarity. Selected distances [Å] and angles [°]: Ni1···Ru1 3.0781(7), O1–O2 1.404(5), Ru1–O1 2.000(3), Ru1–O2 2.027(3), Ru1–S1 2.406(1), Ru1–S2 2.393(1), Ni1–S1 2.179(1), Ni1–S2 2.171(1), Ni1–N1 1.968(4), Ni1–N2 1.966(4); Ni1-S1-Ru1 84.17(4), Ni1-S2-Ru1 84.67(4).

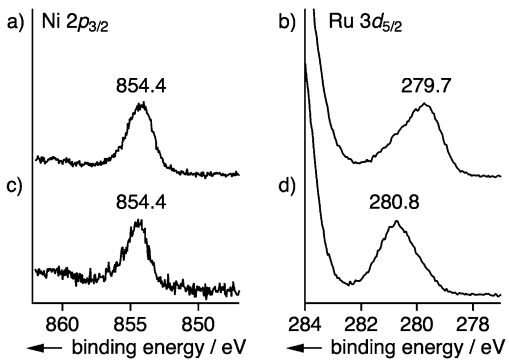


Figure 3. XPS spectra of a) Ni 2p and b) Ru 3d regions for complex [**2a**](NO_3). XPS spectra of c) Ni 2p and d) Ru 3d regions for complex [**2b**](NO_3).

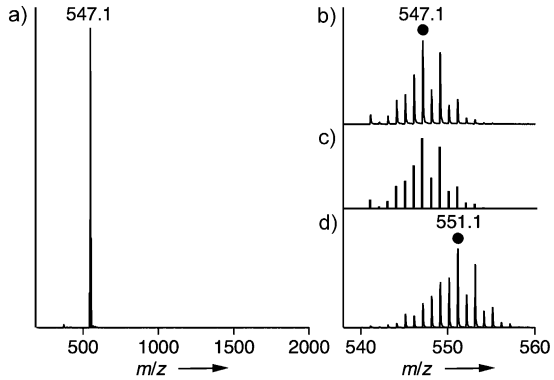


Figure 4. a) Positive-ion ESI mass spectrum of complex [**2b**](NO_3) in water. b) The signal at *m*/*z* 547.1 corresponds to complex [**2b**]$^+$. c) Calculated isotopic distribution for complex [**2b**]$^+$. d) Positive-ion ESI mass spectrum of complex [^{18}O-labeled **2b**](NO_3) in water.

This drastic change in reactivity towards H_2 and O_2 arose from the electronic properties of the aromatic ligands. The negatively charged pentamethylcyclopentadienyl ligand has a stronger electron-donating ability than the neutral hexamethylbenzene ligand, which presumably facilitated the Ru^{II} center in reaching the high-valent Ru^{IV} state.

To confirm the presence of the O_2 adduct, complex [**2b**]-(CF_3SO_3) was isolated and an X-ray structure was successfully obtained (Figure 2). Dark-brown crystals of complex [**2b**](CF_3SO_3) that were suitable for X-ray analysis were obtained by diffusion of diethyl ether into a solution of complex [**2b**](CF_3SO_3) in acetonitrile, which was prepared from the replacement of the counteranion of NO_3^- in complex [**2b**](NO_3) by $CF_3SO_3^-$. The framework of compound **2b** is based around a NiS_2Ru "butterfly" core, in a similar manner to that of compound **1a**, but with minor parametric differences. The Ni1···Ru1 separation was 3.0781(7) Å, which was slightly shorter than that in compound **1a** (3.1611(6) Å). The Ni-S-Ru bite angles were 84.17(4) and 84.67(4)°, slightly smaller than those in compound **1a** (86.81(4) and 87.20(5)°, respectively). The peroxo ligand in compound **2b** was coordinated to the Ru center in a side-on fashion, with an O–O distance of 1.404(5) Å. This distance corresponded to that reported previously for the two-electron-reduction of O_2 into peroxide (O_2^{2-})[16] and was within the range found for the previously-reported [Ru(η^2-O_2)(η^5-C_5Me_5)] complexes (1.316(6)–1.416(5) Å).[17–19] This distance was slightly longer than that found in another dinuclear [NiRu] peroxo complex, [Ni(S_2N_2)Ru(η^2-O_2)(η^5-C_5Me_5)]$^+$ (1.371(8) Å, S_2N_2 = *N*,*N*′-bis(2-mercaptoethyl)-*N*,*N*′-dimethyl-1,3-diaminoethane),[17] and comparable to that reported in a dinuclear [RhRu] peroxo complex, [Rh(Tp*)(μ-SPh)$_2$Ru(η^2-O_2)(η^5-C_5Me_5)] (1.397(3) Å, Tp* = hydrotris(3,5-dimethylpyrazol-1-yl)).[18]

Given the similarities between compound **1a** and [NiFe]-H_2ases, in view of the active site structure and function,[13] we propose that compound **2b** is a good candidate as a mimic of the OBS of [NiFe]H_2ases. Although this O_2 adduct is not unique in the field of such catalysts, it is the first time that its precise relationship pertaining to H_2 activation has been studied, together with an investigation of the relevant chemical properties.

The peroxo ligand in compound **2b** was formed by two-electron reduction of O_2 by compound **2a**. The two electrons came from the Ru^{II} center in compound **2a**, which was investigated by XPS. Figure 3 shows that the binding energies of Ni $2p_{3/2}$ and Ru $3d_{5/2}$ in compound **2a** are 854.4 and 279.7 eV, respectively. The formation of compound **2b** by the oxygenation of compound **2a** resulted in an increase in the binding energy of Ru $3d_{5/2}$ (280.8 eV) and no change in the binding energy of Ni $2p_{3/2}$ (854.4 eV). These results indicated that $Ni^{II}Ru^{II}$ complex **2a** underwent two-electron oxidation by O_2 to form $Ni^{II}Ru^{IV}$–peroxo complex **2b**.[20]

Figure 4 shows a positive-ion ESI mass spectrum of complex [**2b**](NO_3) in water under a N_2 atmosphere. The prominent signal at *m*/*z* 547.1 (I = 100 % in the range *m*/*z* 200–2000) displayed a characteristic isotopic distribution that matched well with the calculated isotopic distribution for complex [**2b**]$^+$. To establish the origin of the peroxo ligand of compound **2b**, ^{18}O-labeled **2b** was synthesized by the reaction of compound **2a** with $^{18}O_2$ in water. ESI-MS results showed that the signal at *m*/*z* 547.1 was shifted to *m*/*z* 551.1. This result indicated that the two ^{18}O atoms from gaseous $^{18}O_2$ had been incorporated into the ^{18}O-labeled **2b**.

Peroxo complex **2b** was formed within 1 second by exposure of compound **2a** to O_2 in water at 25 °C. This rapid conversion of compound **2a** into compound **2b** resulted in the growth of an absorption band at 390 nm; kinetic studies of the oxygenation of compound **2a** were subsequently performed at −20 °C in CH_3CN. The reaction of compound **2a** with excess O_2 in O_2-saturated CH_3CN at −20 °C obeyed pseudo-first-order kinetics with respect to compound **2a** ($v = k_{obs}$[**2a**]) over 5 half-lives, as monitored by UV/Vis spectroscopy (Figure 5). The rate constant (k_{obs}) was determined as $k_{obs} = 1.2 \times 10^{-3}$ s^{-1}.

Following the isolation of the OBS model compound (**2b**), we turned to study its ability to oxidize an external

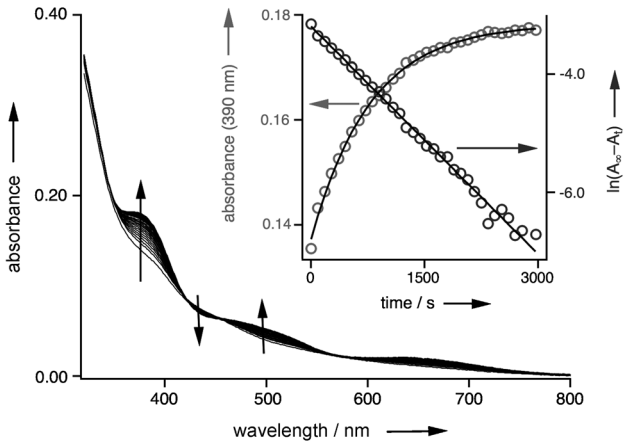


Figure 5. Changes in the UV/Vis spectra during the reaction of complex [**2a**](NO_3) (7.1 μM) with O_2 in O_2-saturated CH_3CN at −20 °C. Inset: time profile of the absorbance at 390 nm and first-order kinetic plots for the change in absorbance at 390 nm of complex [**2a**](NO_3).

substrate. We found that compound **2b** was capable of oxidizing *para*-hydroquinone in water (pH 2.0, under a N_2 atmosphere) into the corresponding *para*-benzoquinone in quantitative yield. Analysis of the kinetic data for the oxidation of *para*-hydroquinone was performed by monitoring the decrease in the absorption band of compound **2b** at 390 nm, which revealed that the reaction of compound **2b** with 10 equivalents of *para*-hydroquinone in water (pH 2.0, 15 °C) followed pseudo-first-order kinetics with respect to compound **2b** ($v = k_{obs}[\mathbf{2b}]$) over 5 half-lives (Figure 6). The rate constant (k_{obs}) was obtained as a slope of the linear first-order plot ($k_{obs} = 2.4 \times 10^{-3}\ s^{-1}$).

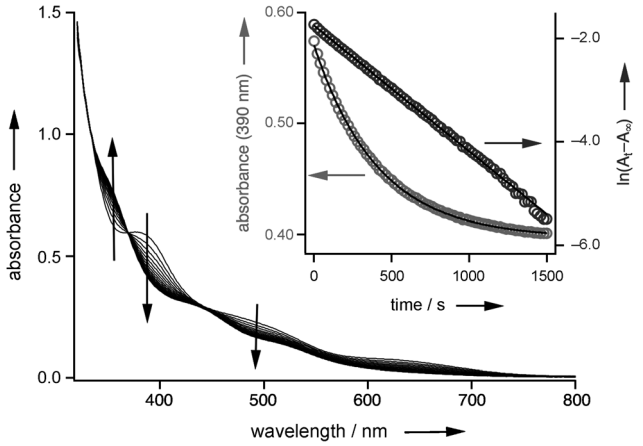


Figure 6. Changes in the UV/Vis spectra for the reaction of complex [**2b**](NO$_3$) (21 μM) with 10 equivalents of *p*-hydroquinone in water (pH 2.0, 15 °C). Inset: time profile of the absorbance at 390 nm and first-order kinetic plots for the change in absorbance at 390 nm of complex [**2b**](NO$_3$).

Compound **2b** was able to acquire electrons and protons from *para*-hydroquinone and $NaBH_4$ in water to regenerate into the initial Ni-SIa model compound (**2a**; Scheme 2).[21] This recovery process was monitored by ESI-MS. The signal at *m*/*z* 547.1, which was derived from compound **2b**, completely disappeared following the addition of 10 equivalents of *para*-hydroquinone and $NaBH_4$ in water, with a concomitant appearance of a signal at *m*/*z* 515.2, which was derived from compound **2a** (pH 2.0, 15 °C, under a N_2 atmosphere, 30 min, Figure 7). Exposure of the regenerated compound **2a** to O_2 led again to the formation of compound **2b**.

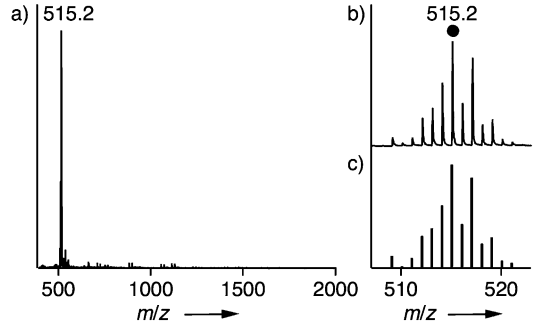
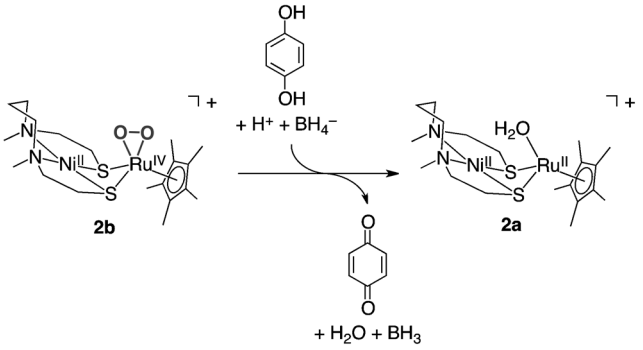


Figure 7. a) Positive-ion ESI mass spectrum obtained from the reaction of complex [**2b**](NO$_3$) with 10 equivalents of *p*-hydroquinone and $NaBH_4$ in water (pH 2.0, 15 °C, under a N_2 atmosphere, 30 min). b) Signal at *m*/*z* 515.2 fragment [**2a**−H_2O]$^+$. c) Calculated isotopic distribution for fragment [**2a**−H_2O]$^+$.



Scheme 2. Regeneration of the Ni-SIa model complex (**2a**) from the OBS model complex (**2b**) by acquiring electrons and protons from *p*-hydroquinone and $NaBH_4$ in water.

These results allowed us to construct a cycle of O_2 activation for [NiRu] biomimetic models of O_2-tolerant [NiFe]-H_2ases (Figure 8). Exposure of the Ni-SIa model compound (**2a**) to O_2 in water to form the OBS model compound (**2b**) corresponded thereby to the deactivation step proposed for natural [NiFe]H_2ases. The deactivated species (**2b**) then ob-

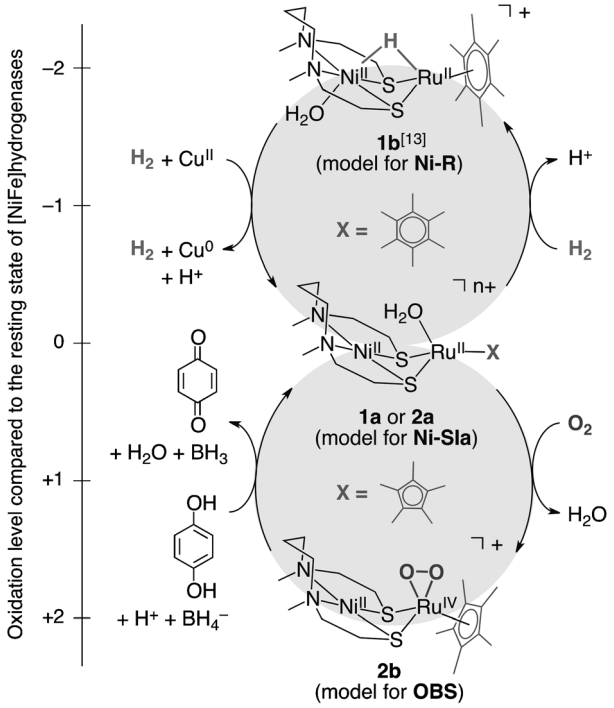


Figure 8. The electron-transport O_2-tolerant mechanism with [NiRu] biomimetic models for [NiFe]H_2ases. **1a**: X = C_6Me_6, *n* = 2; **2a**: X = C_5Me_5, *n* = 1.

tained electrons and protons to return to Ni-SIa species (**2a**); this step corresponded to the recovery step.

Whilst we required two closely related, but different, ligands for our system to follow both cycles, natural O_2-tolerant enzymes can accomplish a similar change in electron donation by simply switching the conveyance of electrons from a proximal {Fe-S} cluster. Hence, although we had to construct our H_2- and O_2-activating systems from different starting materials, the system as a whole was a good mimic for the ability of native enzymes to switch between different activating modes of its active site.

Conclusions

The σ-donating ability of two closely related ligands in [NiFe]H_2ase mimics determined whether the [NiRu] catalyst was H_2-activating, to form a reactive hydride, or O_2-activating, to form an active [NiRu]–peroxo complex. This behavior was considered to mimic the ability of certain [NiFe]-H_2ases to switch between H_2 and O_2 activation by enabling/disabling electron transport from {Fe-S} clusters. The O_2 activation cycle proceeded through a peroxo adduct, and we propose that a similar adduct may be found in O_2-tolerant [NiFe]H_2ases. These findings are expected to be of use in further investigating the properties of natural [NiFe]H_2ases and in designing future catalysts for the controlled activation of H_2 and O_2.

Experimental Section

Materials and Methods

All experiments were carried out under a N_2 atmosphere by using standard Schlenk techniques and a glove box. CH_3CN was distilled over CaH_2 under a N_2 atmosphere prior to use. Mono-Ni^{II} complex [Ni^{II}L] (L = *N,N′*-dimethyl-*N,N′*-bis(2-mercaptoethyl)-1,3-propanediamine),[14] mono-Ru^{II} complex [$Ru^{II}(\eta^5\text{-}C_5Me_5)(CH_3CN)_3$]$(NO_3)$,[15] $Ni^{II}Ru^{II}$–aqua complex [$Ni^{II}LRu^{II}(H_2O)(\eta^6\text{-}C_6Me_6)$]$(NO_3)_2$ ([**1a**]$(NO_3)_2$),[13] and $Ni^{II}Ru^{II}$–hydride complex [$Ni^{II}(H_2O)L(\mu\text{-}H)Ru^{II}(\eta^6\text{-}C_6Me_6)$]$(NO_3)$ ([**1b**]-(NO_3))[13] were prepared according to literature procedures. D_2O was purchased from Cambridge Isotope Laboratories, Inc., $^{18}O_2$ was purchased from SHOKO CO., LTD., and water was purchased from Wako Pure Chemical Industries, Ltd.; these chemicals were used without further purification.

^{1}H NMR spectra were recorded on a JEOL JNM-AL300 spectrometer at 25 °C. ^{1}H NMR experiments (D_2O) were performed in a NMR tube (diameter = 1.5 mm), with [D_4]3-(trimethylsilyl)propionic-2,2,3,3 acid sodium salt (TSP, 100 mM) as the reference compound (the methyl protons or the carbon resonance were set at 0.00 ppm). ESI-MS data were obtained by using a JEOL JMS-T100LC AccuTOF mass spectrometer. IR spectra of solid compounds (KBr disks) were recorded on a Thermo Nicolet NEXUS 8700 FTIR instrument from 650 to 4000 cm^{-1}, by using 2 cm^{-1} standard resolution at 25 °C. UV/Vis spectra were recorded on an Otsuka Electronics photodiode array spectrometer MCPD-2000 with an Otsuka Electronics optical fiber attachment (light path length 0.80 cm). Elemental analysis was obtained on a PerkinElmer 2400II series CHNS/O analyzer. The oxidation states of the metal centers were determined by X-ray photoelectron spectroscopy (XPS), by using a Nissan Arc PHI 5800 system with an Al/Mg dual-anode X-ray source. Binding energies were collected with a C 1s binding energy of the carbon atoms of the ligand in the specimens as 284.5 eV.[22] The pH (pD) value of the solution was adjusted to 2.0 by using 10 mM HNO_3/water (DNO_3/D_2O) and was determined with a pH meter (TOA, HM-20J) that was equipped with a pH combination electrode (TOA, GST-5725C). pD values were corrected by adding 0.4 to the observed values (i.e., pD = pH meter reading + 0.4).[23]

*[$Ni^{II}LRu^{II}(H_2O)(\eta^5\text{-}C_5Me_5)$]$(NO_3)$ ([**2a**](NO_3))*

A solution of a Ni^{II} complex ([Ni^{II}L], 280 mg, 1.0 mmol) in water (25 mL) was added to a solution of the Ru^{II} complex [$Ru^{II}(\eta^5\text{-}C_5Me_5)(CH_3CN)_3$]$(NO_3)$ (420 mg, 1.0 mmol) in water (25 mL) under a N_2 atmosphere to afford a black solution. After stirring for 2 h, the solvent was removed under reduced pressure to yield complex [**2a**](NO_3) as a black powder. The powder was washed with a small amount of water (2.0 mL) and dried in vacuo (yield: 85%, based on [$Ru^{II}(\eta^5\text{-}C_5Me_5)(CH_3CN)_3$]$(NO_3)$). ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = 1.68–1.89, 1.94–2.10, and 2.88–2.95 (m, 14H; CH_2), 2.16 (s, 15H; $C_5(CH_3)_5$), 2.69 ppm (s, 6H; NCH_3); ESI-MS (in water): *m/z* (%) 515.2 [**2a**$-H_2O$]$^+$, 200–2000 (100); FTIR (KBr disk): $\tilde{\nu}$ = 3430 (O–H), 2895 (aliphatic C–H), 1625 (aromatic C=C), 1460 (aromatic C=C), 1431 (aromatic C=C), 1384 cm^{-1} (NO_3); elemental analysis calcd (%) for $C_{16}H_{37}N_3O_4S_2NiRu$: C 38.33, H 6.26, N 7.06; found: C 38.49, H 6.31, N 7.26.

*[$Ni^{II}LRu^{IV}(\eta^2\text{-}O_2)(\eta^5\text{-}C_5Me_5)$]$(NO_3)$ ([**2b**](NO_3))*

Complex [**2a**](NO_3) (66 mg, 0.11 mmol) was dissolved in water (30 mL) and stirred under an O_2 atmosphere for 2 h to afford a dark-brown solution. The solvent was evaporated to yield complex [**2b**](NO_3) as a dark-brown powder (yield: 96%, based on [**2a**](NO_3)). ^{1}H NMR (300 MHz, in D_2O, 25 °C): δ = 2.03–2.45, 2.73–2.83, and 3.40–3.50 (m, 14H; CH_2), 1.62 (s, 15H; $C_5(CH_3)_5$), 2.72 ppm (s, 6H; NCH_3); ESI-MS (in water): *m/z* (%) 547.1 [**2b**]$^+$, 200–2000 (100); FTIR (KBr disk): $\tilde{\nu}$ = 3420 (O–H), 2917 (aliphatic C–H), 1629 (aromatic C=C), 1459 (aromatic C=C), 1430 (aromatic C=C), 1384 cm^{-1} (NO_3); elemental analysis calcd (%) for $C_{16}H_{36}N_3O_{5.5}S_2NiRu$ [**2b**]$(NO_3)\cdot 0.5H_2O$: C 36.90, H 5.87, N 6.79; found: C 36.98, H 5.80, N 7.09.

*[$Ni^{II}LRu^{IV}(\eta^2\text{-}^{18}O_2)(\eta^5\text{-}C_5Me_5)$]$(NO_3)$ ([^{18}O-labeled **2b**](NO_3))*

Isotope-labeled complex [^{18}O-labeled **2b**](NO_3) was obtained in the same manner as described for the preparation of complex [**2b**](NO_3) except that $^{18}O_2$ was used instead. ESI-MS (in water): *m/z* (%) 551.1 [^{18}O-labeled **2b**]$^+$, 200–2000 (100).

*Typical Procedure for the Regeneration of Complex [**2a**](NO_3) from Complex [**2b**](NO_3)*

To a solution of complex [**2b**](NO_3) (30 mg, 50 μmol) in water (3.0 mL) at pH 2.0 was added *p*-hydroquinone (55 mg, 0.50 mmol) and the resulting solution was stirred for 1 h under a N_2 atmosphere to afford a red-brown solution. The addition of $NaBH_4$ (19 mg, 0.50 mmol) to this solution afforded a black solution of complex [**2a**](NO_3) with gas evolution. This reaction was monitored by ESI-MS and by ^{1}H NMR and UV/Vis spectroscopy. The yield of complex [**2a**](NO_3) was 20%, based on complex [**2b**](NO_3), which was determined by ^{1}H NMR spectroscopy, using 1,4-dioxane as an internal standard in D_2O.

*Quantitative Analysis of p-Benzoquinone from the Reaction of Complex [**2b**](NO_3) with p-Hydroquinone by ^{1}H NMR Spectroscopy*

To a solution of complex [**2b**](NO_3) (4.5 mg, 7.4 μmol) in D_2O (1.0 mL) at pD 2.0 was added *p*-hydroquinone (8.1 mg, 74 μmol) under a N_2 atmosphere. The resulting solution was stirred for 30 min. The yield of *p*-benzoquinone as an oxidized product was 95%, based on complex [**2b**]-(NO_3), which was determined by ^{1}H NMR spectroscopy, using 1,4-dioxane as an internal standard in D_2O (*p*-benzoquinone: δ = 6.83 ppm (s, 4H; C_6H_4)).

*Kinetic Measurements of the Reaction of Complex [**2a**](NO_3) with O_2*

The reaction of complex [**2a**](NO_3) with O_2 in O_2-saturated CH_3CN at −20 °C under an O_2 atmosphere was followed by UV/Vis spectroscopy (390 nm). O_2 gas was bubbled through CH_3CN (6.9 mL) in a Schlenk flask (10 mL) at −20 °C for 30 min to give an O_2-saturated CH_3CN solu-

tion. To the resulting solution was added a solution of complex [**2a**](NO_3) in CH_3CN (0.50 mM, 0.10 mL). The final concentration of complex [**2a**](NO_3) was 7.1 μM. The rate constant (k_{obs}) was determined by a least-squares curve fit ($k_{obs} = 1.2 \times 10^{-3}\ s^{-1}$).

Kinetic Measurements of the Reaction of Complex [2 b](NO$_3$) with p-Hydroquinone

The reaction of complex [**2b**](NO_3) with *p*-hydroquinone in water (pH 2.0) at 15 °C under a N_2 atmosphere was followed by UV/Vis spectroscopy (390 nm). A solution of *p*-hydroquinone (15 mM, 0.10 mL) in water was added at 15 °C to water (pH 2.0, 6.8 mL) in a Schlenk flask (25 mL). To the resulting solution was added a solution of complex [**2b**](NO_3) in water (1.5 mM, 0.10 mL). The final concentrations of complex [**2b**](NO_3) and *p*-hydroquinone were 21 μM and 210 μM, respectively. The rate constant (k_{obs}) was determined by a least-squares curve fit ($k_{obs} = 2.4 \times 10^{-3}\ s^{-1}$).

X-ray Crystallographic Analysis

Dark-brown crystals of complex [**2b**](CF_3SO_3) that were suitable for X-ray analysis were obtained by diffusion of Et_2O into a solution of complex [**2b**](CF_3SO_3) in CH_3CN, which was prepared from the replacement of the counteranion of NO_3^- in complex [**2b**](NO_3) by $CF_3SO_3^-$ by addition of $NaCF_3SO_3$ in CH_3CN. Measurements were made on a Rigaku/MSC Saturn CCD diffractometer with graphite monochromated $Mo_{K\alpha}$ radiation ($\lambda = 0.71070$). All calculations were performed by using the teXsan crystallographic software package of the Molecular Structure Corporation. CCDC 785544 ([**2b**](CF_3SO_3)) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre at www.ccdc.cam.ac. uk/data_request/cif. Crystal data: $C_{20}H_{35}F_3N_2NiO_5RuS_3$; $M_r = 696.45$; orthorhombic; $a = 13.0153(21)$ Å, $b = 14.4222(23)$ Å, $c = 27.9367(47)$ Å; $V = 5244.0(15)$ Å^3; space group *Pbca*; $Z = 8$; $T = 123.2$ K; 38506 reflns measured; 5971 unique reflns ($R_{int} = 0.109$); $R_1 = 0.048$; $wR = 0.123$; GOF on F^2: 0.998.

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