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A Ni(I)Fe(II) analogue of the Ni-L state of the Active Site of the [NiFe] Hydrogenases

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[NI(L\textsuperscript{1})Fe\textsuperscript{3+}(BuNC)\textsubscript{3}](PF\textsubscript{6})\textsuperscript{2-} is a robust Ni\textsuperscript{II} complex that undergoes a reversible one-electron reduction. Spectroscopic and theoretical studies show that [NI(L\textsuperscript{1})Fe\textsuperscript{3+}(BuNC)\textsubscript{3}]\textsuperscript{2-} is an unprecedented Ni\textsuperscript{II} species that reproduces the electronic configuration of the Ni-L state of the [NiFe] hydrogenases.

The [NiFe]-hydrogenases catalyse the two-electron inter-conversion of two protons and molecular hydrogen.\textsuperscript{1} The nature of the Ni-Fe heterobimetallic active site in these enzymes is now established; the Ni centre is co-ordinated by two terminal and two bridging cysteinate donors, which co-ordinate to a Fe centre that is also bound by one carbonyl and two cyanide ligands (Scheme 1). Catalytic H\textsubscript{2} cleavage is associated with changes in the formal oxidation state of the Ni centre while the Fe centre remains in the Fe\textsuperscript{II} state during turnover.\textsuperscript{2c} Three key states have been identified in a catalytic cycle (Scheme 1): Ni-SI, Ni-R and Ni-C. Thus, H\textsubscript{2} reacts with Ni-SI and undergoes heterolytic cleavage to form Ni-R which contains a bridging H\textsuperscript{+} ligand. A co-ordinated Cys ligand may act as an initial proton acceptor for the accompanying proton before its transfer to other bases (B) about the active site.\textsuperscript{3} The removal of an electron generates the EPR active S = \frac{1}{2} Ni-C state, which can be converted to an EPR-active S = \frac{3}{2} Ni\textsuperscript{II} state (Ni-L) following the photolysis at low temperatures.\textsuperscript{4} Previously, Ni-L had not been viewed as being catalytically relevant given the conditions required for its formation. However, recent in situ IR spectroelectrochemical studies have demonstrated that Ni-L may be generated reversibly in the dark under turnover conditions.\textsuperscript{5} Therefore, the regeneration of the Ni-SI state could occur either directly from the Ni-C state, via the concerted transfer of an electron and a proton, or by oxidation of the Ni-L state. These studies, together with previous DFT calculations,\textsuperscript{5} open up the possibility that separate proton and electron transfer events may be associated with the regeneration of the Ni-SI state from Ni-C, and that these steps may involve the Ni-L state (Scheme 1).

Despite the large number of diamagnetic Ni\textsuperscript{II}Fe\textsuperscript{III} complexes that have been prepared as analogues of the [NiFe] hydrogenases,\textsuperscript{6} the syntheses of paramagnetic analogues have proven to be more challenging.\textsuperscript{7} Several Ni\textsuperscript{II}Fe\textsuperscript{III}, Ni\textsuperscript{III}Fe\textsuperscript{III}, Ni\textsuperscript{II}Fe\textsuperscript{II} and Ni\textsuperscript{II}Fe\textsuperscript{II} centres have been reported,\textsuperscript{6c,7,8} none of which have succeeded in reproducing the crucial Ni\textsuperscript{II}Fe\textsuperscript{III} and Ni\textsuperscript{II}Fe\textsuperscript{III} states found for the [NiFe] hydrogenases. For example, [(dppe)Ni(μ-pdt)Fe(CO)\textsubscript{3}](BF\textsubscript{4})\textsuperscript{2-} [dppe = 1,2-bis(diphenylphosphino)ethane, pdt = propane-1,2-dithiolate] possesses a Ni\textsuperscript{II}Fe\textsuperscript{III} configuration with spin density localized principally on the Fe centre and [(dppe)Ni(μ-pdt)Ru(cymene)]\textsuperscript{9} possesses a NiRu\textsuperscript{II} centre rather than the biologically more relevant Ni\textsuperscript{II}Fe\textsuperscript{III} unit. Given the renewed focus on the role of Ni-L, we report the characterisation of a Ni\textsuperscript{II}Fe\textsuperscript{II} complex ([1]\textsuperscript{1+} as an analogue of this state. [1]\textsuperscript{1+} is prepared from the reversible, one-electron reduction of the parent complex [1]\textsuperscript{2+} (Fig. 1). Our assignment of [1]\textsuperscript{1+} as a Ni\textsuperscript{II}Fe\textsuperscript{II} centre represents the first analogue of the Ni-L form of the [NiFe] hydrogenases to feature Ni and Fe centres with electronic configurations that mirror those proposed for Ni-L.

Treatment of a solution of [Ni(L\textsuperscript{1})] \textsubscript{0} \textsuperscript{1+} = N\textsubscript{2}N'-diethyl-3,7-diazanonane-1,9-dithiol\textsuperscript{10} in acetonitrile with FeCl\textsubscript{3} followed by the addition of four equivalents of BuNC and NH\textsubscript{2}PF\textsubscript{6} affords [Ni(L\textsuperscript{1}N\textsubscript{2}S)Fe\textsuperscript{3+}(BuNC)\textsubscript{3}](PF\textsubscript{6})\textsubscript{2} ([1](PF\textsubscript{6})\textsubscript{2}). [1](PF\textsubscript{6})\textsubscript{2} is stable at room
temperature in air as a solid and in acetonitrile solution for at least 48 h, as monitored by IR spectroscopy. The crystallographic characterization of [1](PF₆)₂-MeCN shows the Ni centre in an approximate square-planar N₄S₂ environment comprised of four BuNC ligands and two S donors derived from [Ni(L)₁] (Fig. 1). The NiN₂S₂ fragment retains the structural features of the [Ni(L)₁] precursor, the most significant difference being a smaller S(1)-Ni(1)-S(2) angle (81.76(2)° in [Ni(L)₁] relative to that in [1] (84.20(2)°). This difference may reflect the steric demands of the co-ordinated [Fe(BuNC)₄]²⁻ fragment in [1]. The equatorial BuNC ligands defined by C(2) and C(3) bind to Fe(1) in an essentially linear mode with Fe(1)-C-N angles of 175.3(2)° and 175.1(2)°, respectively. In contrast the axial BuNC ligands, defined by C(1) and C(4), coordinate in a bent geometry with Fe(1)-C-N angles of 170.0(2) and 172.9(2)°, respectively. The Ni(1)-C(1) and Ni(1)-C(4) distances (2.951(2) and 4.164(2) Å, respectively) are significantly greater than the Fe(1)-C(1) and Fe(1)-C(4) distances (1.890(2) and 1.888(2) Å, respectively). Thus, the axial BuNC ligands do not appear to adopt bridging modes between the Ni and Fe centres in [1] and the non-linear binding mode of these ligands about Fe(1) may result from inter- and intra-molecular interactions due to crystal packing (Fig. S1). The Ni(1)-Fe(1) distance (2.9898(7) Å) compares well with that in the inactive oxidised form of [NiFe] hydrogenase from Desulfovibrio gigas (2.9 Å) and is significantly longer than that found in the Ni-R form from Desulfovibrio vulgaris Miyazaki F (2.57 Å) that both contain Ni and Fe in formal M⁰ oxidation states.

The cyclic voltammogram of [1](PF₆)₂, recorded at 298K in MeCN containing 0.2 M [N(Bu₄)₄][BF₄] as supporting electrolyte, shows a reduction process at E₁/₂ = -1.39 V vs FC/Fc that is reversible over the range of scan rates employed in the experiment (20 – 300 mVs⁻¹, Figs. S2 and S3). The cyclic voltammogram of [Ni(L)₁] recorded under the same conditions reveals a reduction process at E₁/₂ = -2.35 V vs FC/Fc (Fig S4), assigned to the reduction of [Ni(L)₁] to the formal Ni⁰ state on the basis of comparisons with previously reported NiN₂S₂ complexes possessing similar co-ordination spheres.⁴ The shift of ca. +1 V for the reduction of [1](PF₆)₂ relative to that of [Ni(L)₁] is consistent with the formation of a Lewis base Lewis acid adduct between [Ni(L)₁] and [Fe(BuNC)₄]²⁻; ca. +0.5 V shifts in potential have been observed previously for (NiN₂S₂)W(CO)₄ relative to their parent NiN₂S₂ complexes.¹³ UV/Vis spectrotoclectrochemistry indicates that [1] decomposes at temperatures above 273 K and that cooling to 243K is required to ensure the quantitative regeneration of [1] (Fig. S5, Table S1). On cooling to 243 K the cyclic voltammogram of [1](PF₆)₂ becomes electrochemically irreversible (Fig. S6) and the controlled potential electrolysis of [1](PF₆)₂ at -1.6 V vs FC/Fc at 243K confirms that a one-electron reduction process accompanies the formation of [1]⁺. The cyclic voltammograms of [1](PF₆)₂ and [1]⁺ at 243K exhibit similar profiles confirming the stability of [1]⁺ under the conditions and timescale of the experiment (Fig. S7).

The IR spectra of [1](PF₆)₂ and [1]⁺ in MeCN solution are shown in Fig. 2. Each spectrum exhibits four bands assigned to the [Ni(L)₁]⁻ fragment (Fig. S5, Table S1). On cooling to 243K the cyclic voltammogram of [1](PF₆)₂ exhibits four bands at frequencies typical of isocyanide ligands bound in a terminal mode to transition metal centres.¹⁴ The overall shift of the bands to lower frequencies following the reduction of [1] to [1]⁺ is consistent with an increase in the electron density about the Ni-Fe core and a corresponding increase in n-back-donation into the BuNC units. A C-N stretching band at 1857 cm⁻¹ in the IR spectrum of [1]⁻ in MeCN solution suggests that one terminal, apical BuNC ligand moves to a bridging mode between the Ni and Fe centres (Fig. 2) following the reduction of [1]⁻; a similar bridging mode is found in [Fe₂(pdt)(MeNC)₃](PF₆)₂ where one MeNC ligand bridges between the two Fe centres.¹⁵

The X-band EPR spectrum of electrochemically generated [1]⁻ recorded at 77 K in MeCN / 0.2 M [N(Bu₄)₄][BF₄] (Fig. 3a) shows striking similarities to those of Ni₃W₂S₂ complexes (S = thiolato, thioether or sulfonato, N = amine donors; g∥ = 2.18 – 2.25; g₁ = 2.057-2.071), generated by chemical reduction of their Ni⁰ counterparts, and, crucially, is substantially different to those of Fe centres including [Fe(CO)₉(PPh₃)₃]⁻ (g∥ = 2.053, g₁ = 2.090, g₂ = 2.001)¹⁷ and [(dppe)Ni(μ-pdt)Fe(CO)₄]⁻ (g∥ = 2.052, g₁ = 2.050, g₂ = 2.005 for one isomer).⁷ Thus, the EPR spectroscopic data are consistent with a formal NiFe⁰ unit in [1]⁻ where the Ni⁻ centre adopts a d⁸, S = ½ configuration in which the unpaired electron resides in d-orbital orientated in the equatorial plane of the Ni₃W₂S₂ unit with associated spin Hamiltonian parameters g∥ > g₁ > g₂ > g₃. In contrast, the Ni-L form of the [NiFe] hydrogenases is characterised by a rhombic EPR spectrum (g₁ = 2.30, g₂ = 2.12 and g₃ = 2.05)¹⁹ that may be viewed as resulting from the re-
hybridization of the Ni $d_{x^2}$ and $d_{y^2}$ orbitals in Ni-C which one hybrid contributes to a Ni-Fe bond.\textsuperscript{20} The UV/vis spectrum of \[1\] (Table S1 and Fig. S5) shows bands at 400 (3900), 490 (1760), 520 (1500), 598 (1100) and 720 nm (380 M$^{-1}$ cm$^{-1}$) that are consistent with those in the UV/vis spectra of other well-defined Ni complexes.\textsuperscript{21}

In order to support the NiFe$^+\text{II}$ assignment proposed for \[1\], we conducted density functional theory (DFT) calculations on the full structures of \[1\].\textsuperscript{30} The calculated structure of \[1\]\textsuperscript{30} resembles the principal features of the experimentally determined structure (Fig. 1); the average Fe-C distances are ca. 0.02 Å shorter and the Ni-S and Fe-S distances are ca. 0.04 Å longer in the calculated structure of \[1\] (Table S3). The principal differences between the calculated and experimental structures are (i) a relaxation of the dihedral angle defined by the S(1)-Ni(1)-S(2) and S(1)-Fe(1)-S(2) planes [117.94(3)$^\circ$ and 128.3$^\circ$, in the calculated and experimental structures, respectively], (ii) an increase in the Ni(1)-Fe(1) distance of ca. 0.2 Å in the calculated relative to the experimental structure of \[1\] (Ni(1)-Fe(1) = 3.209 Å and 2.989(6) Å for the calculated and experimental structures, respectively, Table S2), and (iii) an increase in the Fe-C(1)-Ni-C(1)-C(2) angles for the axial BuNC ligands defined by C(1) and C(4) (Table S2). The uncalculated IR spectrum of \[1\] possesses four bands in the C-N stretching region at 2216, 2183, 2169 and 2139 cm$^{-1}$ that compare well with the experimental stretching frequencies (Fig. 2). Thus, the close correspondence between the calculated and experimental structures, and IR spectra suggest that the DFT calculations provide a reasonable description of the geometric and electronic structure of \[1\].\textsuperscript{30} The composition of the HOMO in \[1\] shows that it is largely metal-centred [59.3% Ni $d_{x^2}$, 6.0% Ni $d_{y^2}$, 9.7% Fe $d_{z^2}$, 1.2% Fe $d_{yz}$, S 25.8%, N(5)+N(6) 1.2%, Fig. S9] The Mayer bond order\textsuperscript{24} between the Ni(1) and Fe(1) centres (0.04) derived from the DFT calculations suggests there is no formal metal-metal bond in \[1\].\textsuperscript{25} Overall the description of the electronic structure of \[1\] is consistent with an S = 0 NiFe$^+$ centre in \[1\].\textsuperscript{26}

The calculated structure of \[1\] suggests that significant changes in geometry about the Ni and Fe(1) centres accompany the reduction of \[1\] (Fig. S8(b) and Table S3) These include a marked decrease in the dihedral angle between the S(1)-Ni(1)-S(2) and S(1)-Fe(1)-S(2) planes (94.1$^\circ$ and 128.3$^\circ$, for \[1\] and \[1\], respectively) and a shortening of the Ni(1)-Fe(1) distance [Ni(1)-Fe(1) = 2.616 Å and 3.209 Å for \[1\] and \[1\], respectively], which compares well with that calculated for models of the active site of the Ni-L form.\textsuperscript{20} On the reduction of \[1\] one axial BuNC ligand, defined by C(1), moves to a bridging mode between the Fe(1) and Ni(1) centres with Fe(1)-C(1) and Ni(1)-C(1) distances of 1.942 and 2.018 Å, respectively. The adoption of a bridging mode for this ligand is accompanied by a significant bend in the backbone of the ligand [C(1)-N(1)-C(5) = 140.9$^\circ$], which is commonly observed for bridging isocyanides.\textsuperscript{25} The calculated IR spectrum for \[1\] shows three intense C-N stretches at 2179, 2140 and 2121 cm$^{-1}$ for the terminal isocyanides and a single band at 1872 cm$^{-1}$ for the C-N stretching mode of the bridging BuNC ligand. This calculated spectrum shows close correspondence to the experimental IR spectrum of \[1\] (Fig. 2) and strongly supports a structural rearrangement in which a terminal BuNC ligand moves to a bridging mode on the reduction of \[1\].\textsuperscript{27}

This structural rearrangement may also underpin the differences in profiles of the cyclic voltammograms of \[1\] recorded at 298 K (Fig. S2) and 243K (Fig. S6). The rate of the suggested structural rearrangements for \[1\] may be slowed at 243K with the consequent loss of electrochemical reversibility for the \[1\] process at 243K. We are unable to determine the precise mechanism that gives rise to the voltammetric profile i.e. whether electron transfer precedes structural rearrangement or vice versa. However, the results of the UV/vis spectrophotometric experiments clearly show that the process is chemically reversible at 243K over the timescale of this experiment.

The SOMO of \[1\] possesses 60.8% Ni $d_{xy}$, 1.3% Ni $d_{yz}$, 1.1% Ni $d_{z^2}$ S 21.7%, N(5)+N(6) 10.2% character and is essentially localized on the NiBuNC ligand at 243K with the consequent loss of electrochemical reversibility for the NiBuNC ligand. This calculated spectrum shows close correspondence to the experimental IR spectrum of \[1\].\textsuperscript{28} We note that the DFT calculations underestimate the largest g-shift ($\beta_{\text{g}} = 2.174$ calc vs 2.079, Table S2) reproduce the approximately axial nature of the frozen solution EPR spectrum of \[1\] (Fig. 3a). We note that the DFT calculations underestimate the largest g-shift ($\beta_{\text{g}} = 2.174$ calc vs 2.079, Table S2) reproduce the approximately axial nature of the frozen solution EPR spectrum of \[1\]. (Fig. 3a).

The calculated NiFe$^+$ Mayer bond order increases from 0.04 in \[1\] to 0.20 in \[1\] indicating the development of a Ni-Fe interaction but not a direct bond. In contrast, DFT calculations on models of the Ni-L state possess Ni-Fe bond orders of ca. 0.40 supporting the formation of a metal-metal bond in these centres.\textsuperscript{20} The absence of a formal Ni-Fe bond in \[1\] is not surprising given the additional fourth BuNC ligand in the co-ordination sphere of Fe$^+$ which occupies a bridging position between the Ni and Fe centres; this site is vacant in structures proposed for Ni-L.

Ni-L reacts with CO and converts to the paramagnetic Ni-CO state which features a CO ligand bound to the Ni$^+$ centre.\textsuperscript{27} Thus, we examined the reactivity of \[1\] and \[1\] towards CO. Whereas \[1\] does not react with CO, a solution of \[1\] chemically generated from \[1\] ready reads with CO at 243 K, as
monitored by IR spectroscopy (Fig. S10). Several new bands develop in the C-O and C-N stretching region and the frozen solution EPR spectrum exhibits multiple low field features (Fig. S10) suggesting the formation of multiple products, which proved intractable.

In conclusion, [1\(^{1+}\)] has been prepared and structurally characterized as its [1\(^{1+}\)](PF\(_6\)) salt. The electrochemical one-electron reduction of [1\(^{1+}\)] generates paramagnetic [1\(^{1+}\)] which has been characterized by IR, UV/vis and EPR spectroscopies. DFT calculations reproduce the principal features of the IR spectrum of [1\(^{1+}\)] and, in contrast to Ni-L, [1\(^{1+}\)] does not contain a formal Ni-Fe bond. Rather the formation of [1\(^{1+}\)] may be associated with a structural rearrangement that incorporates a bridging BuNC ligand between the Ni and Fe centres. The frozen solution X-band EPR spectrum of [1\(^{1+}\)] and DFT calculated spin Hamiltonian parameters are consistent with a SOMO that is largely localized at the NiN\(_2\) core in a Ni d\(_x\) orbital. Thus, the experimental and theoretical data supports the assignment of [1\(^{1+}\)] to a mixed-valence NiFe\(^{2+}\) state. In this respect [1\(^{1+}\)] represents the first example of an Ni-Fe analogue of the active site of the [NiFe] hydrogenases that reproduces the formal oxidation and spin states of the metal centres in the Ni-L form.

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**Notes and references**


