# A square-planar Ni(II) complex with an $N_2S_2$ donor set similar to the active centre of nickel-containing superoxide dismutase and its reaction with superoxide†

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**The structure around the metal centre of a Ni(II) complex with an $N_2S_2$ square-planar geometry, 1, prepared as a model compound of the NiSOD active site was drastically changed upon addition of potassium superoxide ($KO_2$).**

Ni-containing superoxide dismutase (NiSOD) catalyzes disproportionation of superoxide anion to dioxygen and hydrogen peroxide through the following reactions:[1]

$$\text{Ni(II)} + O_2^- + 2H^+ \rightarrow \text{Ni(III)} + H_2O_2 \qquad \text{(i)}$$

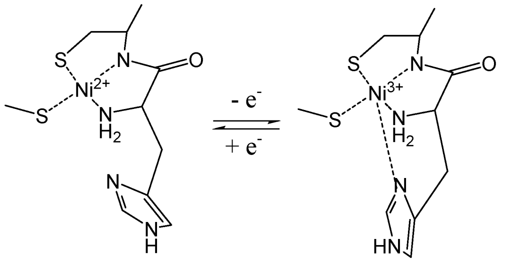
$$\text{Ni(III)} + O_2^- \rightarrow \text{Ni(II)} + O_2 \qquad \text{(ii)}$$

The crystal structure of the active site has been reported for both the Ni(II) and Ni(III) states. In the Ni(II) state, the central metal ion has a square-planar geometry with ligands provided by one amine nitrogen of the terminal histidine, one amide nitrogen of the peptide backbone, and two cysteine thiolates at equatorial positions.[2] When the Ni(II) state is oxidized to the Ni(III) state, the coordination structure is converted to square-pyramidal geometry and the imidazolyl group of the terminal histidine moves to an axial position and ligates to the Ni centre with a relatively long ligand–metal distance of ~2.5 Å (Scheme 1).[2] Evidence for this coordination structure is provided by an EPR spectral feature typical of a $d_{z^2}$ ground state with triplet hyperfine splitting in the $g_\parallel$ region. On the other hand, the EPR spectrum of the H1Q mutant of NiSOD (H1Q-NiSOD) also exhibits triplet hyperfine splitting in spite of the lack of the terminal histidine imidazole group.[3] As a result of this observation, the structure of the Ni(III) active site of NiSOD remains controversial.

In this work, we describe a new $N_2S_2$-type square-planar Ni(II) complex (**1**) as an active site model of NiSOD, and identify interesting structural behaviour exhibited during the reaction of **1** with superoxide.

The crystal structure of **1**, obtained by vapour diffusion of $Et_2O$ into an acetone solution of **1**, is shown in Fig. 1 together with selected bond lengths and angles.‡ The Ni(II) centre of **1** has $C_2$ symmetry with square-planar geometry. Ligands are provided by two amine nitrogens and two thioether sulfurs in the equatorial plane. The bond lengths of Ni–N (1.931(4) Å) and Ni–S (2.197(1) Å) are consistent with those of diamine- or dithioether-type square-planar Ni(II) complexes with three five-membered chelate rings.[4–7] Complex **1** has an absorption band characteristic of Ni(II) complexes with a square-planar structure at 474 nm ($\varepsilon$/$M^{-1}$ $cm^{-1}$ 210) in MeOH. This band is lower in energy than those of related $N_2S_2$-type Ni(II) complexes reported previously; $[\text{Ni(emi)}]^{2-}$ (diamide-dithiolate-type, 430 nm (340)),[4] $[\text{Ni(BEAAM)}]^-$ (monoamide-monoamine-dithiolate-type, 461 nm (290)),[5] [Ni(bmmp-dmed)] (diamine-dithiolate-type, 480 nm),[6] $[\text{Ni(ema)}\cdot(\text{Me})_2]$ (diamide-dithiolether-type, 416 nm (360))[7] and reduced NiSOD (450 nm (480)).[8] The Ni(II) complex **1** produces reversible and quasi-reversible redox potentials corresponding to Ni(I)/(II) and Ni(II)/(III) couples at −0.68 and 1.71 V (*vs.* NHE), respectively. The potential for Ni(II)/(III) is much higher than the analogous potential of other Ni complexes with diamide-dithiolate- (−0.50 V), diamide-dithioether- (1.24 V), diamine-dithiolate- (1.04 V ($E_{pa}$)), or monoamide-monoamine-dithiolate-donor sets (−0.04 V).[4–7] The absorption band observed in the lower energy region and the higher value of the redox potential indicate that the ligating atoms are weaker donors than those of typical $N_2S_2$-type Ni(II) complexes.

The reaction of the Ni(II) complex **1** with $NaBH_4$ produces an EPR spectrum typical of a low-spin $d_{x^2-y^2}$ ground state ($g_\parallel = 2.25$, $g_\perp = 2.07$) as shown in Fig. 2(A). This implies that the Ni(II) complex **1** is reduced to the Ni(I) state while retaining square-planar geometry. After exposure of this solution to air, the EPR spectrum is converted to a spectrum typical of a $d_{z^2}$ ground state with triplet hyperfine splitting in the $g_\parallel$ region (Fig. 2(B)). This EPR spectral change suggests that the Ni(I) complex is oxidized by $O_2$ to a low-spin Ni(III) state.



**Scheme 1** Schematic structures of the NiSOD active site in the reduced (left) and oxidized (right) states.

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† Electronic supplementary information (ESI) available: Experimental section and additional figures. CCDC 757339. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b925755a

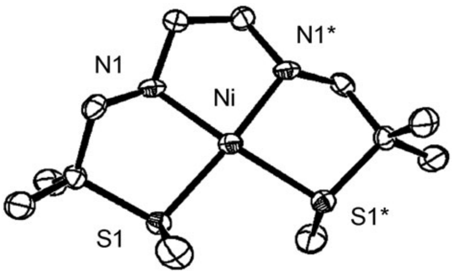


**Fig. 1** ORTEP drawing of the cation moiety of **1** with thermal ellipsoids drawn at 50% probability. The hydrogen atoms and counter-anions (perchlorate) are omitted for clarity. The atoms with and without an asterisk are related by the crystallographic twofold axis. Selected bond distances (Å) and angles (°): Ni–N 1.924(3), Ni–S 2.1981(10), N1–Ni–N1* 87.26(13), N1–Ni–S1* 173.97(8), N1–Ni–S1 88.60(9), N1*–Ni–S1* 88.60(9), S1–Ni–S1* 95.85(4).

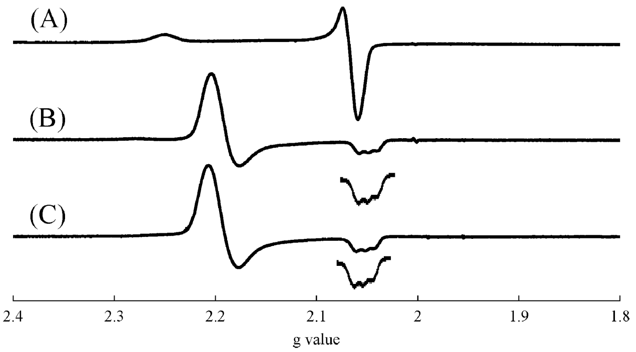
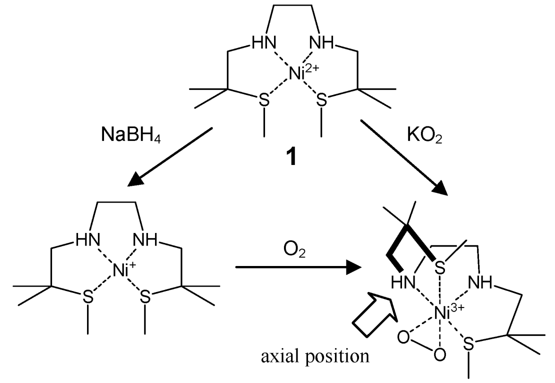


**Fig. 2** EPR spectra observed (A) after reduction of **1** using $NaBH_4$, (B) after exposure of the solution to air, and (C) by reaction between **1** and $KO_2$

The same EPR spectrum is also observed as a result of the reaction between the Ni(II) complex **1** and almost the same equivalent amount of potassium superoxide ($KO_2$) in MeOH (Fig. 2(C)). Furthermore, both reaction products have the same absorption maxima at 300 nm. These spectroscopic observations indicate that this reaction yields the same product as that of the reaction of the Ni(I) complex and $O_2$. This reaction corresponds to step (i) of the superoxide dismutation mechanism of NiSOD. The $\nu$(O–O) stretching band in the resonance-Raman spectrum of the reaction product is observed at 923 $cm^{-1}$ (Fig. S4, ESI†), a value similar to the $\nu$(O–O) stretching band of side-on Ni-dioxygen species.[9]

The triplet hyperfine splittings ($|A_{\parallel}| = 1.5$ mT) are observed in the $g_{\parallel}$ region in spite of the fact that no additional exogenous nitrogen donors are present. These findings suggest that a nitrogen atom from the supporting ligand coordinates to the axial position of the Ni(III) centre, thereby altering the coordination structure of Ni(II) complex **1** from the square-planar structure to another distinct coordination structure upon oxidation.



**Scheme 2** The reaction scheme of complex **1** treated with $NaBH_4$ and subsequent addition of $O_2$ (left), and that of **1** with $KO_2$.

The EPR spectral behavior indicates that the coordination geometry is dramatically altered as a result of the reaction. The reactions between the Ni(I) species and $O_2$ and between the Ni(II) species and $KO_2$ should both produce side-on Ni(III)-peroxo species as shown in Scheme 2. One of the two thioether sulfur atoms moves to the *trans* position of one of the peroxide oxygen atoms. One of two amine nitrogen atoms may be changed from the equatorial position to the axial position of the new equatorial plane. The high structural flexibility of the amine nitrogen site may permit such a structural change.

Although the EPR spectrum of a d$_{z^2}$ ground-state Ni(III) complex with triplet hyperfine splitting in the $g_{\parallel}$ region is observed only in cases where the axial position of the Ni(III) centre is occupied by an exogenous nitrogen-containing ligand with $I = 1$,[4,10–13] the structural changes described above are permissible. The Ni(III) ion has a rigid square-planar structure with imine or amide nitrogen ligands. This structure permits exogenous ligands to bind only at the axial positions.[4,10–12] Therefore, higher structural flexibility of the ligands is necessary in order to enable the drastic structural change to occur as shown in Scheme 2. It is expected that the oxidized product of **1** is more flexible than other Ni(III) complexes, because there is no rigid moiety. In fact, such "kicked-up" structures were previously reported for Cu(II) and Ni(II) complexes containing a thioether sulfur.[14–17] In these previous reports, the thioether sulfurs result in "kicked-up" structures when a stronger ligand is provided.

As mentioned previously, the oxidized form of the H1Q mutant of NiSOD also exhibits triplet hyperfine splitting in the $g_{\parallel}$ region in EPR spectra in spite of the lack of the terminal histidyl imidazole.[3] The Ni(II) complex with a flexible $N_2S_2$-type square-planar structure indicates another possibility to explain this controversial result. From the crystal structure of native NiSOD, it is estimated that H1Q- and native NiSODs may also have higher flexibility similar to that of complex **1**.[2] We therefore propose that the thiolate sulfur atom of Cys6 on the equatorial plane of active centre of H1Q- and native NiSOD can be kicked up to the axial position when a stronger donor such as superoxide anion is present.

In summary, we have prepared a new square-planar Ni(II) complex **1** with an $N_2S_2$ donor set and characterized it spectroscopically and electrochemically. The complex is oxidized by $KO_2$ to Ni(III) in MeOH. The same product is also obtained in a reaction between the reduced product of **1** and $O_2$. The oxidized state of **1** exhibits an EPR signal with triplet hyperfine splitting in the $g_{\parallel}$ region in spite of the lack of an exogenous nitrogen donor. These findings suggest that the active site structures of H1Q- and native NiSOD undergo significant changes upon oxidization.

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## Notes and references

‡ Crystallographic data for **1**: $C_{12}H_{28}Cl_2N_2NiO_8S_2$, $M_r$ = 522.09, monoclinic, $C2/c$, $a$ = 17.864(4), $b$ = 8.569(2), $c$ = 13.909(3) Å, $\beta$ = 107.176(3)°, $V$ = 2034.2(8) Å$^3$, $Z$ = 4, $D_c$ = 1.705 g cm$^{-3}$, unique refls. ($R_{int}$) 2297 (0.019), $R$ ($I > 2\sigma(I)$)/$R_w$ (all reflections) = 0.0452/0.1452, GOF = 1.018.

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