# Nitrosylation of 2Fe–2S and 4Fe–4S Models for Iron–Sulphur Redox Proteins

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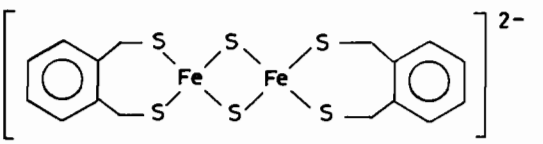
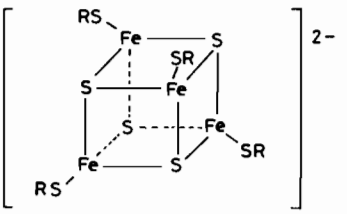
Paramagnetic iron–nitrosyl complexes, characterised by usually anisotropic $g$ values around 2.03 have been observed [1–3] in extracts of rat liver following the administration of specific chemical carcinogens: similarly $g$ = 2.03 complexes have been found [4, 5] in extracts from many of the organs of several species of experimental animals maintained on a normal diet supplemented only by sodium nitrite and iron(II) sulphate. In all of these studies the identification of the $g$ = 2.03 species as complexes of general type $[Fe(NO)_2L_2]^{+x}$ was far from definitive and was based primarily upon a comparison of their EPR spectra with those of the complexes originally observed [6] by Phillips *et al.* in the reactions of iron(II) salts with nitric oxide in the presence of anionic ligands. In each case the $g$ = 2.03 complexes were assigned [1–5] structures of type $[Fe(NO)_2(SR)_2]^-$ where R remained unidentified. Closely related [7] to the paramagnetic species $[Fe(NO)_2(SR)_2]^-$ are the diamagnetic dinuclear complexes $Fe_2(NO)_4(SR)_2$: the methyl ester $Fe_2(NO)_4(SMe)_2$ has been isolated from vegetable sources, and it has been implicated in the causation of oesophageal cancer [8–11]. The $Fe_2S_2$ core of this naturally occurring ester is presumably derived from an iron–sulphur redox protein, as also are the iron–sulphur groupings of the $g$ = 2.03 complexes, suggesting that nitrosylation of the iron–sulphur cores of such proteins can proceed readily, at least *in vivo*. In the present communication, we report evidence that the nitrosylation of synthetic models for natural iron–sulphur proteins is readily achieved under mild conditions.

Reaction of either of the models for 2Fe–2S and 4Fe–4S redox proteins **I** and **II** respectively, as their tetraethylammonium salts, with sodium nitrite in aqueous alkaline medium, followed by acidic work-up [12] gave the sodium salt $Na[Fe_4S_3(NO)_7]$ in isolated, purified yields of 44% and 38% respectively, based upon total iron. For many other reactions in which the anion $[Fe_4S_3(NO)_7]^-$ is formed from precursors of the same or different nuclearity, we have previously demonstrated [7] that paramagnetic mononuclear complexes of type $[Fe(NO)_2L_2]^{+x}$ having $g$ = *ca.* 2.03, (where L = solvent, or RS or $H_2S$), are crucial intermediates. Since in particular the mononuclear complex $Fe(NO)_2(SH)(SH_2)$ is a direct precursor of $[Fe_4S_3(NO)_7]^-$ in a number of reactions [7], providing a direct route for fragmentation and spontaneous re-assembly in reactions involving a change of nuclearity, it seemed probable that the *in vitro* (and hence by implication the *in vivo*) nitrosylation of natural and model iron–sulphur clusters might also proceed via paramagnetic mononuclear $g$ = 2.03 complexes.




**I**




**II**

Consistent with this view, we observed that reaction of **I**, as its $Et_4N^+$ salt, in DMF solution with nitric oxide yielded an equimolar mixture of two paramagnetic iron nitrosyl complexes, **III** and **IV**. Complex **III** was characterised by $g$ = 2.033, $A(^{14}N)$ = 2.4 G(2N), $A(^1H)$ = 4.09(2H), and hence was identified as the known [7] solvo-complex $[Fe(NO)_2(DMF)_2]^+$: complex **IV** was characterised by $g$ = 2.207, $A(^{14}N)$ = 2.5 G(2N), $A(^1H)$ = 4.5 G(2H), $A(^1H)$ = 4.4 G(1H), and thus has [7] the constitution $Fe(NO)_2(DMF)(SR)$ where the group SR has two hydrogens on the α-carbon: *i.e.* the di-thio ligand of **I** must in **IV** be acting either as a monodentate ligand or as a bridging ligand between two well-separated irons. Furthermore, addition of excess iodide to this mixture gave the characteristic reaction [7, 13] of iron(–I) nitrosyl complexes, forming as the sole paramagnetic product $[Fe(NO)_2I_2]^-$, characterised [13] by its eleven line e.p.r. spectrum, with $g$ = 2.079, $A(^{127}I)$ = 20.6 G. The tetra-iron cluster **II**, again as its $Et_4N^+$ salt, showed no reaction with NO/DMF at ambient temperature, but on a brief heating again yielded a mixture of paramagnetic mononuclear nitrosyls, forming $[Fe(NO)_2I_2]^-$ upon addition of excess iodide.

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The most plausible external source of nitrosyl groups in biological systems is nitrite, introduced either directly or by reduction of nitrate. Reaction of **I**, as its $Et_4N^+$ salt with either $(PPN)^+NO_2^-$ or $NaNO_2$ in DMF at ambient temperature rapidly yielded a new mononitrosyl complex having $g$ = 2.031, $A(^{14}N)$ = 12.3 G(1N), which in turn was rapidly converted to the known [7] $Fe(NO)_2(SH)(SH_2)$. A similar reaction of **II** yielded initially a mononitrosyl complex, whose spectrum was rapidly replaced by that of a mixture of species. In each case, the products from **I** and **II** were converted to $[Fe(NO)_2I_2]^-$ by addition of iodide.

In contrast to the action of both nitric oxide and nitrite ion on these model clusters, the N-nitrosamine $Me_2NNO$ did not react over a period of days with a solution of **I** in DMF to yield detectable paramagnetic iron nitrosyls: furthermore, subsequent addition of iodide gave no $[Fe(NO)_2I_2]^-$, indicating that no transfer of nitrosyl groups from the nitrosamine nitrogen to iron had occurred. This should be compared with the observed [8] transfer of nitrosyl groups in the opposite direction: the neutral ester $Fe_2(SMe)_2(NO)_4$ reacts with amines to yield N-nitrosamines.

In conclusion, we have demonstrated that both 2Fe–2S and 4Fe–4S models for iron–sulphur redox proteins are nitrosylated by either nitric oxide or nitrite to yield both paramagnetic and diamagnetic iron nitrosyl species: while nitric oxide yields a paramagnetic complex $[Fe(NO)_2(solv)(SR)]$ still containing the terminal alkanethiolate ligand, nitrite leads via $Fe(NO)_2(SH)(SH_2)$ to $[Fe_4S_3(NO)_7]^-$.

## Acknowledgement

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