The isolated iron–molybdenum cofactor of nitrogenase catalyses hydrogen evolution at high potential

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The isolated cofactor of nitrogenase FeMoco catalyses hydrogen evolution at the high potential associated with the FeMoco$^{ox/semi\text{-}red}$ couple ($E°$ = −280 mV *vs.* NHE , $C_6F_5S^-$ ligated form); analysis of the current–potential dependence of the catalysis suggests a mechanism involving rate-determining loss of H_2 from an FeMoco(H)$_2$ ^{red} intermediate (k = 3 s^{-1}); the relatively slow kinetics of this step may be related to an obligatory role for hydridic intermediates in substrate reductions by nitrogenase.

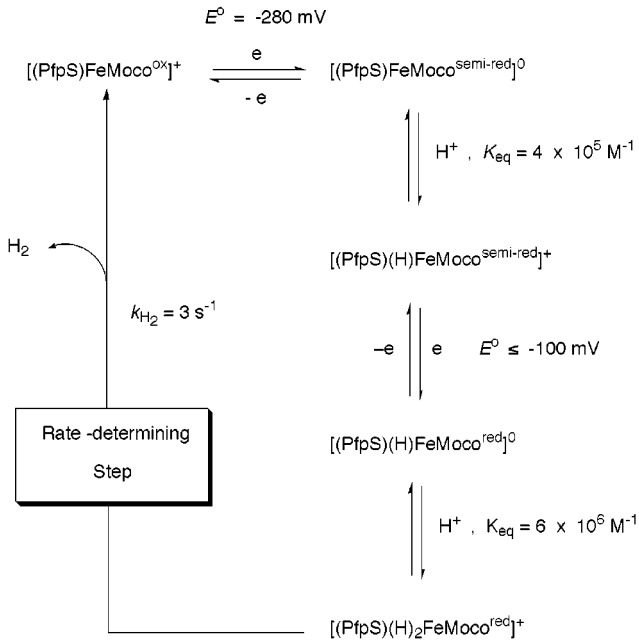
Catalysis of proton reduction to dihydrogen is an intrinsic property of the nitrogenase system. In the absence of molecular nitrogen (or other reducible substrates) turnover of the enzyme results in hydrogen evolution which is believed to be associated with successive 1e/1H$^+$ transfers to the resting-state redox level of the molybdenum–iron protein.[1] The centre within this protein at which proton reduction takes place is almost certainly[2] the {$MoFe_7S_9$}-cluster which has been shown to have the structure depicted in Scheme 1 when the protein is in the resting-state.[3] Disruption of the protein ligation of the cluster allows extraction into *N*-methylformamide (NMF) of a cofactor (FeMoco) which reactivates mutant apo-protein.[4] Spectroscopic, analytical and other evidence indicate that FeMoco retains the structure of the protein bound {$MoFe_7S_9$}-cluster but with the histidine ligand replaced by coordination of a solvent molecule to Mo and the cysteinyl ligand at the capping Fe replaced by an anion, probably *N*-methylformamidate.[5] Herein we show that FeMoco is capable of electrocatalysing the reduction of protons to dihydrogen at high potential, a process which may directly relate to the enzymic catalysis.

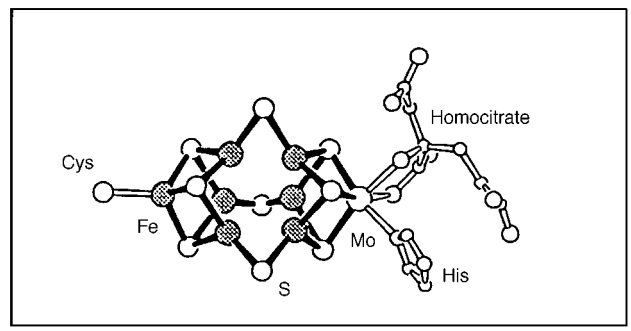
FeMoco was extracted into an NMF–phosphate buffer solution from molybdenum–iron protein that had been isolated from *Klebsiella pneumoniae* using minor modifications of the method described earlier.[6] It was derivatised by pentafluorothiophenolate (PfpS$^-$) ligation at the capping tetrahedral Fe atom,[7] and studied in the oxidised, EPR-silent, form which we shall refer to as {(PfpS)FeMoco}^{ox}. Cyclic voltammetry of {(PfpS)FeMoco}^{ox} showed that the cofactor undergoes a well defined, reversible, diffusion-controlled, single-electron reduction to give {(PfpS)FeMoco}$^{semi\text{-}red}$. This directly parallels earlier observations by Schultz, Newton and coworkers on the thiophenolate derivatised form of the cofactor.[8]

However, pentafluorothiophenol can function both as a ligand to FeMoco and as a source of protons. The pK_a(H_2O) for PfpSH (2.68) is considerably lower than that for PhSH (6.43) and we would expect that this greater acidity of PfpSH also pertains in the NMF system. Fig. 1 shows the effect of increasing [PfpSH]$_{total}$ on the cyclic voltammetry of the {(PfpS)FeMoco}$^{ox/semi\text{-}red}$ couple.† The peak current, I_p^{red}, is enhanced and the reversibility of the system decreased as the concentration of the acid is increased. Fast potential scan-rates (v) restore some reversibility to the system whereas at low v, the current response approaches that expected for a catalytic substrate reduction with the magnitude of I_p^{red} independent of v.

Controlled potential electrolysis of the cofactor (0.5 μmol) was performed at $E_{applied}$ = −550 mV *vs.* SCE (*ca.* −320 mV *vs.* NHE) in a sealed electrochemical cell in the presence of 60 mM of [PfpSH]$_{total}$. The gas-space was sampled after the passage of 4.3 μmol of electrons. Gas chromatographic analysis confirmed the formation of dihydrogen (1.84 μmol, 85%, *ca.* 9 turnovers). An enzyme reconstitution assay[4] of a sample of the catholyte after electrolysis showed that the cofactor was still fully active, *i.e.* the structural integrity of the cofactor was retained.

Fig. 2 shows a plot of I_p^{red} *vs.* [PfpSH]$_{total}$ together with the calculated response based on the mechanism proposed in Scheme 1. At low [PfpSH]$_{total}$ the phosphate buffer neutralises protons and there is little enhancement of I_p^{red}; above [PfpSH]$_{total}$ *ca.* 40 mM the current increases as the semi-





Scheme 1 Mechanism and key constants for hydrogen evolution catalysed by extracted FeMoco. [PfpSFeMoco^{ox}] is assigned an *arbitrary* net charge of 1+ to clarify proton/electron counting. The potentials are quoted *vs.* NHE which is taken to be 242 mV positive of SCE in the NMF electrolyte.[8] The box shows the structure of Femoco bound within the protein in the semi-reduced resting state.[3]

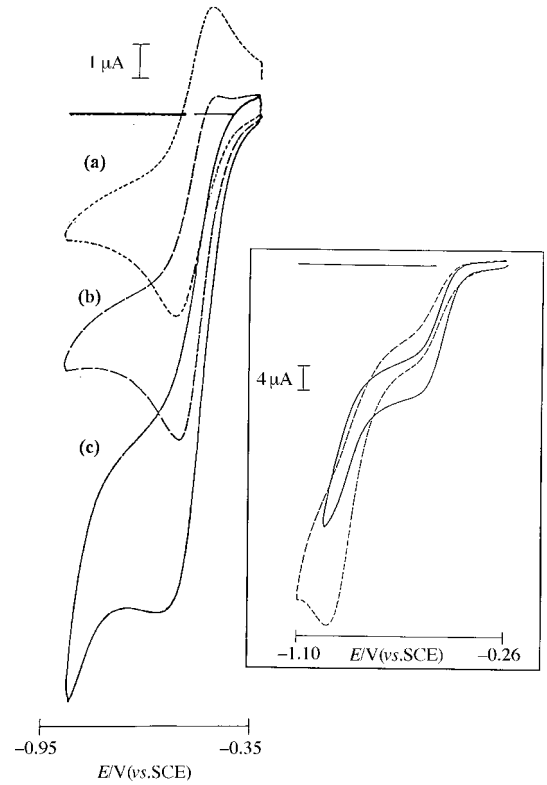


Fig. 1 Effect of adding PfpSH on the cyclic voltammetry of the {(PfpS)FeMoco}$^{ox/semi\text{-}red}$ system. *Conditions*: 1.3 mM FeMoco extract in NMF containing sodium hydrogen phosphate, *ca.* 40 mM; potential scan-rate = 20 mV s^{-1} at a vitreous carbon working electrode of area 0.0707 cm^2; SCE reference and platinum secondary electrodes were employed. Nominal concentration of PfpSH (a) 30 mM (b) 60 mM and (c) 75 mM. Inset: effect of cyanide ($[Et_4N]CN$, 2 equiv.) on the catalytic wave, $[PfpSH]_{total}$ = 135 mM; (—) before addition of cyanide; (- - -) after addition of 2 equiv. CN^-. Note the enhanced reduction of free proton at the more negative potential in the presence of cyanide.

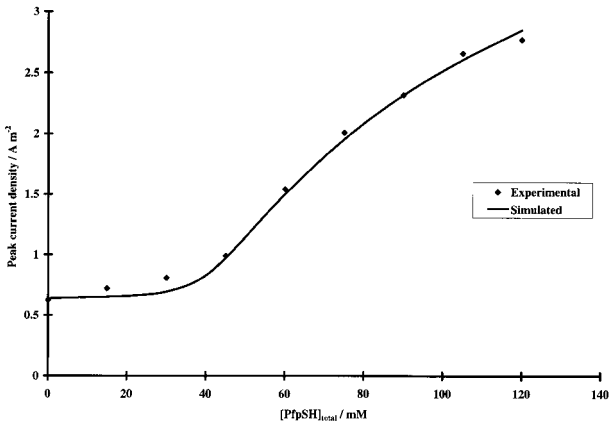


Fig. 2 Plot of I_p^{red} *vs.* $[PfpSH]_{total}$. The points are experimental data, the solid line is the simulated response based upon the mechanism shown in Scheme 1. For the simulation all protonation steps were assumed to be diffusion-controlled; the acid dissociation constant of PfpSH in NMF was taken as 2×10^{-7}; the diffusion coefficient of FeMoco^{ox} used in the simulation was independently measured as 1.65×10^{-6} cm^2 s^{-1} at 293 K; full details of the simulation are supplied as supplementary material (http://www.rsc.org/suppdata/1999/cc/773).

reduced cofactor protonates and is engaged in the catalytic cycle.‡ The rate-determining step in the mechanism involves the irreversible loss of dihydrogen from an hydridic intermediate, {(PfpS)FeMoco$(H)_2$}^{red}; the first-order rate constant (k_{H_2}) for this step is estimated to be 3 s^{-1} at 293 K (Scheme 1).

Accessing reduced states of the cofactor at high potential by protonation may well be essential for the binding of molecular nitrogen, or other substrates, within the enzyme.[1,9] Oxidation by fast release of molecular hydrogen from such states cannot be conducive to efficient catalysis, *viz.* nitrogenase is a 'poor' hydrogenase. The observation that reduced states of isolated FeMoco can be accessed at high potential and that re-oxidation by dihydrogen loss involves a kinetically slow step may be closely linked to the behaviour of the enzyme.

A key feature of hydrogen evolution by nitrogenase is its inhibition by cyanide which is presumed to bind to the iron–molybdenum cluster and decreases electron flux to this centre.[1] Remarkably we also find that cyanide decreases catalytic reduction of protons by isolated cofactor. Fig. 1 (inset) shows the effect of cyanide (2 equiv.) on the catalytic wave. This wave is suppressed and that for direct reduction of proton at the electrode is enhanced. Detailed studies of the electrochemistry of FeMoco in the presence of cyanide, which will be reported elsewhere, show that ligation of this inhibitor to the cofactor shifts the potential of the FeMoco$^{ox/semi\text{-}red}$ couple to a more negative value. This presumably accounts for suppression of electron flux at high potential in both the isolated cofactor and the enzyme system.

In conclusion, we are now beginning to see that the extracted cofactor can engage in electron-transfer chemistry which has some of the attributes of the whole enzyme system : catalytic hydrogen evolution which is kinetically slow and cyanide inhibition of the catalysis. Other parallels are also emerging, for example we have observed the capacity of the cofactor to bind carbon monoxide when the system turns over.[10] In addition, there are reports that acetylene can be catalytically reduced by amalgam reductants in the presence of FeMoco.[11] It is not excessively speculative to suggest that, by suitable modification, it may be possible to achieve the binding and reduction of molecular nitrogen by isolated FeMoco.

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Notes and references

† $[PfpSH]_{total}$ is the concentration of the added thiol if it were undissociated. It corresponds to $[PfpSH]_{total}$ = {[PfpSH] + [$PfpS^-$]}.
‡ The equilibrium constant for protonation of {(PfpS)FeMoco}$^{semi\text{-}red}$ is very similar to that determined for the unmodified cofactor in a related system.[12]

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