The Unusual Reaction of [NiFe]-hydrogenases with Cyanide

Suzannah V. Hexter, Min-Wen Chung, Kylie A. Vincent*
and Fraser A. Armstrong *

Department of Chemistry, University of Oxford, Inorganic Chemistry Laboratory,
South Parks Road, Oxford OX1 3QR, U.K.

Supporting Information

Effect of cyanide when introduced at a low potential.

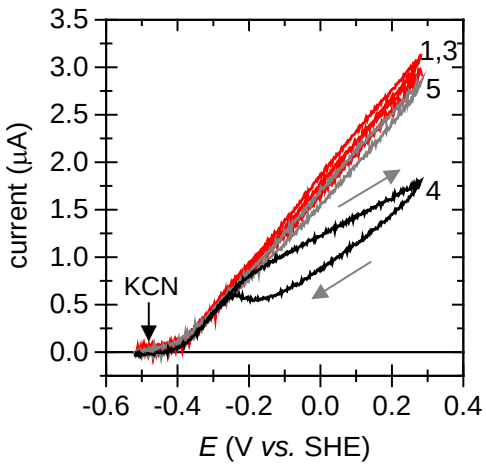

Figure S1: The effect of cyanide on Hyd2 when introduced at –520 mV during a set of cyclic voltammetry scans. The electrode was rotated at 3500 rpm and the H_2 flow rate through the headspace of the cell was set at 500 sccm. Scans prior to the introduction of cyanide are shown in red and those after are shown in shades of grey. The scan number is indicated alongside each plot. Other conditions: pH 7; 25 ^{o}C; scan rate = 4 mV s^{-1}. The concentration of KCN in the cell immediately after injection is 1.4 mM. Gray arrows indicate the direction of the scan.

Measuring the time course of HCN evaporation from the cell.

Spectrophotometric determination of cyanide concentration was completed by using the pyridine-pyrazolone method first proposed by Epstein,[1] but using the modifications later proposed by Nagashima.[2] This method makes use of the König reaction.

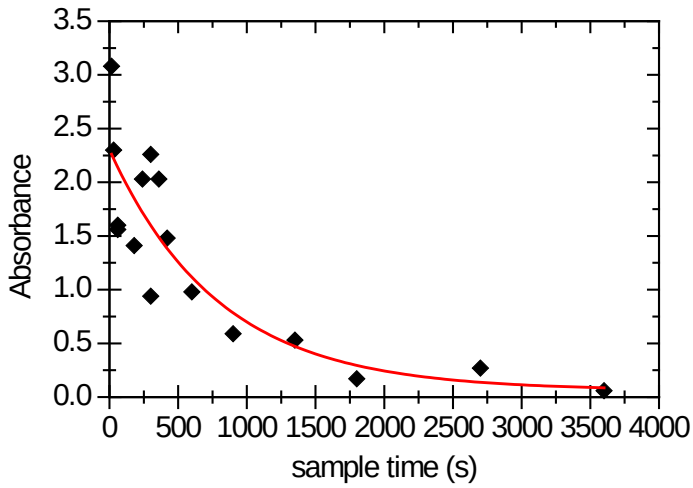

Figure S2: A summary of UV-vis spectroscopic data measuring the decrease in cyanide concentration over time as HCN is flushed out of the electrochemical cell. Other conditions: pH 7; 25 ^{o}C; initial concentration of KCN in the cell (2.15 mL buffer) is 1.4 mM; rate of H_2 flushing through the cell is 500 sccm; electrode rotation rate is 3500 rpm. Fitting the data to a single exponential decay (shown in red) shows that, under these conditions, CN^- is removed from the cell (as HCN) with a half life of approximately 795 s.

Effect of pH on the rate of inactivation of Hyd2 by cyanide.

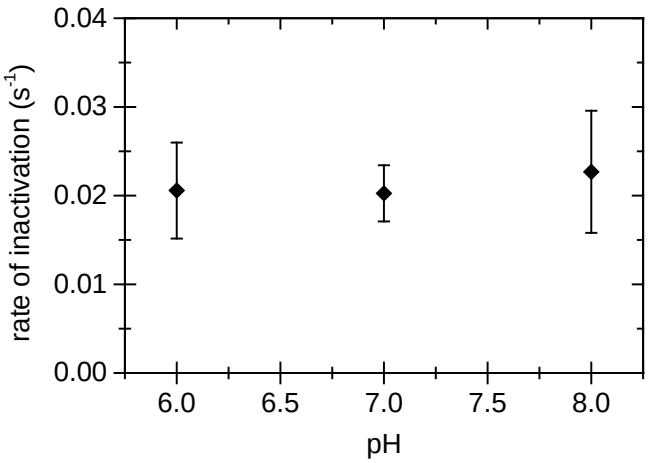

Figure S3: The effect of pH on the rate of cyanide inactivation of Hyd2. An aliquot of KCN solution was injected into the cell resulting in a final concentration of 0.70 mM. The headspace of the cell and the buffer within it were equilibrated to 100% H_2 before starting each experiment. In each case the rate of inactivation was determined by fitting the 'fast' part of the inactivation, accounting for at least the first three half lives, to a single exponential as exemplified in Figure 3A. There was no gas flow though the cell headspace during the experiments, electrode rotation rate = 100 rpm, 25 ^{o}C.

The basis of Equation 1

The mechanism outlined in Scheme 2 leads to a simple mathematical model for fitting the experimental data. Representing the free inactive enzyme (Ni-SI_r) as E, enzyme 'bound' to H_2 (and thus active in the catalytic cycle) as E:H_2 and enzyme bound to cyanide as E:HCN, the approximate concentrations of active species are:

$$\mathrm{E:H_2} \xrightarrow{K_d^{H2}} \mathrm{E + H_2} \qquad \text{S 1}$$

$$\mathrm{E:HCN} \xrightarrow{K_d^{CN}} \mathrm{E + HCN} \qquad \text{S 2}$$

$$K_d^{H2} = \frac{[\mathrm{E}][\mathrm{H_2}]}{[\mathrm{E:H_2}]} \qquad \text{S 3}$$

$$K_d^{CN} = \frac{[\mathrm{E}][\mathrm{HCN}]}{[\mathrm{E:HCN}]} \qquad \text{S 4}$$

The two pathways for inactivation are:

$$\mathrm{E} \xrightarrow{k_1} \mathrm{Ni\text{-}B} \qquad \text{S 5}$$

and

$$\mathrm{E:HCN} \xrightarrow{k_2} \mathrm{Ni\text{-}B} \qquad \text{S 6}$$

Solving for $[\mathrm{E_{tot}}] = [\mathrm{E}] + [\mathrm{E:HCN}] + [\mathrm{E:H_2}]$ S 7

we obtain

$$k_{obs} = \frac{k_1 K_d^{H_2}}{(K_d^{H_2} + [\mathrm{H_2}])} + \frac{k_2 K_d^{H_2}[\mathrm{HCN}]}{(K_d^{CN} + [\mathrm{HCN}])(K_d^{H_2} + [\mathrm{H_2}])} \qquad \text{S 8}$$

References

(1) Epstein, J. *Analytical Chemistry* **1947**, *19*, 272.
(2) Nagashima, S. *Water Research* **1983**, *17*, 833.