A kinetic and thermodynamic understanding of O_2 tolerance in [NiFe]-hydrogenases

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Supporting information

S1. The Rates of O_2 Reaction

As mentioned in the text, not all O_2-reaction rate data fit cleanly to a single exponential process. Data were analyzed by three methods: by fitting the entire current decay to a single exponential, by concentrating on the initial rapid decay and fitting a single exponential to this data, or by fitting to two processes, one fast and one slower. Table S1 summarizes the rates thus obtained; whilst the precise values differ significantly between analyses, the *trends* in these values are consistent between data sets.

Table S1. Detailed analysis of the rates of O_2 reaction, using three alternative methods to extract rate constants from current *vs.* time data. The values in bold are shown in the main text.

Potential (V *vs.* SHE)	+0.192	+0.262	+0.332	+0.402
Rate of O_2 reaction (s^{-1})				
determined by fitting a single exponential curve to all data points	*0.06*	*0.05*	*0.05*	*0.06*
determined by fitting a single exponential curve to only initial, rapid current decay	**0.11**	**0.10**	**0.10**	**0.11**
determined by fitting two exponential curves to all data points, one with a high rate constant, one with a lower rate	*0.13* *0.03*	*0.13* *0.03*	*0.14* *0.03*	*0.15* *0.03*
All values were recorded at pH 5.5, 10 °C				

pH	4.5	5.5	6.5
Rate of O_2 reaction (s^{-1})			
determined by fitting a single exponential curve to all data points	*0.07*	*0.06*	*0.06*
determined by fitting a single exponential curve to only initial, rapid current decay	**0.12**	**0.11**	**0.11**
determined by fitting two exponential curves to all data points, one with a high rate constant, one with a lower rate	*0.15* *0.03*	*0.13* *0.03*	*0.12* *0.03*
All values were recorded at 10 °C, at a constant overpotential (driving force) of +523 mV relative to the thermodynamic H^+/H_2 cell potential at each pH.			

Temperature (°C)	0	10	20	30
Rate of O_2 reaction (s^{-1})				
determined by fitting a single exponential curve to all data points	*0.04*	*0.06*	*0.10*	*0.09*
determined by fitting a single exponential curve to only initial, rapid current decay	**0.06**	**0.11**	**0.19**	**0.31**
determined by fitting two exponential curves to all data points, one with a high rate constant, one with a lower rate	*0.08* *0.02*	*0.13* *0.03*	*0.22* *0.03*	*0.36* *0.02*
All values were recorded at pH 5.5, at a constant overpotential (driving force) of +523 mV relative to the thermodynamic H^+/H_2 cell potential at each temperature.				

S2. The rate of anaerobic inactivation

Typical experiments to measure the rate of anaerobic inactivation are shown in Figure S2. An electrode modified with *Re* H16 MBH was initially held at –0.508 V *vs.* SHE to ensure that all the enzyme was active. The electrode was then stepped to a high potential, causing the enzyme to anaerobically inactivate. Stepping the electrode back to –0.508 V reactivated the enzyme, allowing multiple experiments to be performed on the same enzyme film. The inactivation traces fitted well to a single exponential decay corresponding to an inactivation rate ~0.003 s^{-1}; the rate was independent of the electrode potential in the range 0.242 V – 0.542 V.

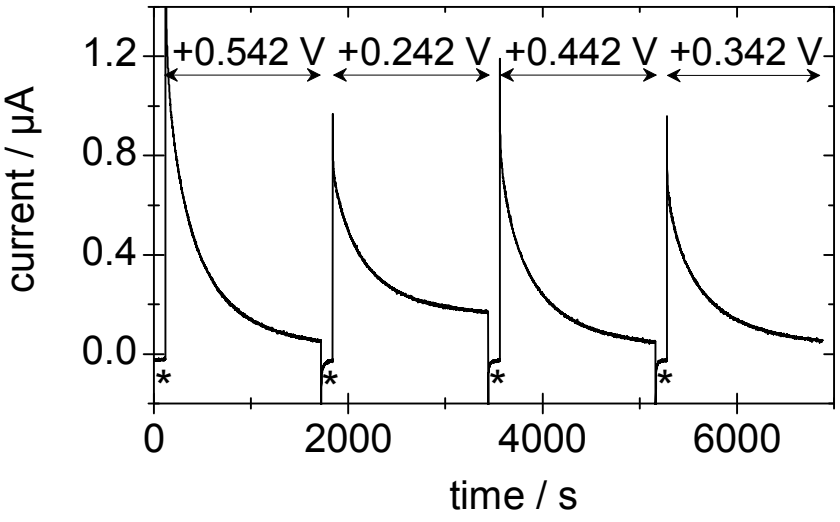

Figure S2. A typical experiment to determine the rate of anaerobic inactivation of *Re* MBH. The electrode potential was stepped between a low value (–0.508 V, indicated *) to activate the enzyme, and a high value (indicated in the figure) at which potential the enzyme anaerobically inactivates. Experimental conditions were: pH 5.5, 10 °C, 100% H_2, electrode rotation rate = 2500 rpm.

S3. The Derivation of Equation 1

Equation 1 was derived as follows, starting from the catalytic scheme shown in the text and reproduced below (Figure S3).

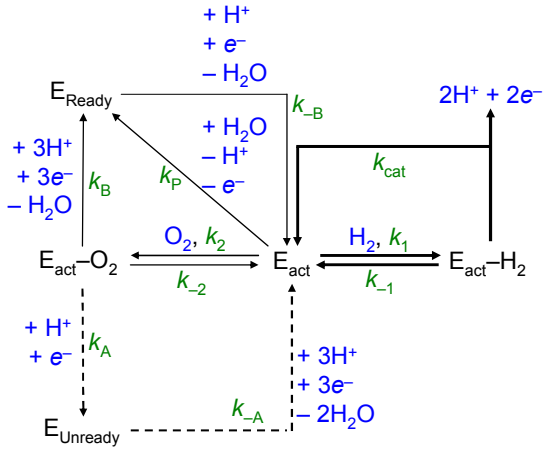

Figure S3. The kinetic scheme used to model O_2 tolerance. *Re* MBH is abbreviated, for clarity, to the single initial 'E', and is described as being in its reduced, active form (E_{act}), forming an adduct with either H_2 or O_2 (E_{act}–H_2 and E_{act}–O_2, respectively) or being in either the Ready state (E_{Ready}) or the Unready ($E_{Unready}$) state.

For ease of notation, we make the following substitutions:

$$\begin{aligned} [\mathrm{E_{act}}] &= \beta & [\mathrm{E_{act}}]_0 &= \beta_0 \\ [\mathrm{E_{act}\text{-}H_2}] &= \alpha & [\mathrm{E_{act}\text{-}O_2}] &= \sigma \\ [\mathrm{E_{Ready}}] &= \varepsilon & [\mathrm{E_{Unready}}] &= \omega \end{aligned} \qquad [1]$$

The steady state approximation is applied to all intermediate species, generating the following expressions for the concentrations of each:

$$\frac{d\alpha}{dt} = k_1[\mathrm{H_2}]\beta - (k_{-1} + k_{cat})\alpha = 0 \quad \Rightarrow \quad \alpha = \frac{k_1[\mathrm{H_2}]\beta}{k_{-1} + k_{cat}} \qquad [2]$$

$$\frac{d\sigma}{dt} = k_2[\mathrm{O_2}]\beta - (k_B + k_A + k_{-2})\sigma = 0 \quad \Rightarrow \quad \sigma = \frac{k_2[\mathrm{O_2}]\beta}{k_B + k_A + k_{-2}} \qquad [3]$$

$$\frac{d\varepsilon}{dt} = k_{\mathrm{P}}\beta + k_{\mathrm{B}}\sigma - k_{-\mathrm{B}}\varepsilon = 0 \qquad \Rightarrow \qquad \varepsilon = \frac{k_{\mathrm{P}}\beta + k_{\mathrm{B}}\sigma}{k_{-\mathrm{B}}} \qquad [4]$$

$$\frac{d\omega}{dt} = k_{\mathrm{A}}\sigma - k_{-\mathrm{A}}\omega = 0 \quad \Rightarrow \qquad \omega = \frac{k_{\mathrm{A}}\sigma}{k_{-\mathrm{A}}} \qquad [5]$$

The total concentration of active enzyme is given by the difference between the initial concentration of enzyme, $[E_{act}]_0 = \beta_0$, and the concentration of all intermediate species, *i.e.*:

$$\beta = \beta_0 - \alpha - \sigma - \varepsilon - \omega \qquad [6]$$

The Michaelis constant for H_2, $K_M^{H_2}$, is defined as:

$$K_{\mathrm{M}}^{\mathrm{H_2}} = \frac{k_{-1} + k_{\mathrm{cat}}}{k_1} \qquad [7]$$

and an inhibition constant for O_2 inhibition, $K_I^{O_2}$, is defined as:

$$K_{\mathrm{I}}^{\mathrm{O_2}} = \frac{k_{\mathrm{B}} + k_{\mathrm{A}} + k_{-2}}{k_2} \qquad [8]$$

From equation 2:

$$\alpha = \frac{[\mathrm{H_2}]}{K_{\mathrm{M}}^{\mathrm{H_2}}}\left(\beta_0 - \alpha - \sigma - \varepsilon - \omega\right) \qquad [9]$$

and thus

$$\alpha = \frac{\beta_0 - \sigma - \varepsilon - \omega}{1 + \dfrac{K_{\mathrm{M}}^{\mathrm{H_2}}}{[\mathrm{H_2}]}} \qquad [10]$$

Substituting in, we obtain:

$$\alpha = \frac{\beta_0 - \sigma - \left(\dfrac{k_{\mathrm{P}}\beta + k_{\mathrm{B}}\sigma}{k_{-\mathrm{B}}}\right) - \left(\dfrac{k_{\mathrm{A}}\sigma}{k_{-\mathrm{A}}}\right)}{1 + \dfrac{K_{\mathrm{M}}^{\mathrm{H_2}}}{[\mathrm{H_2}]}} \qquad [11]$$

Solving for σ leads to

$$\sigma = \frac{\beta_0 - \alpha\left(1 + \frac{K_M^{H_2}}{[H_2]} + \frac{k_P}{k_{-B}}\frac{K_M^{H_2}}{[H_2]}\right)}{1 + \frac{k_B}{k_{-B}} + \frac{k_A}{k_{-A}}} \quad [12]$$

Similarly, from equation 3, we obtain:

$$\sigma = \frac{[O_2]}{K_I^{O_2}}\left(\beta_0 - \alpha - \sigma - \varepsilon - \omega\right) \quad [13]$$

and thus

$$\sigma = \frac{\beta_0 - \alpha - \varepsilon - \omega}{1 + \frac{K_I^{O_2}}{[O_2]}} \quad [14]$$

From Equation 10, by substituting in Equations 4 and 5, we obtain:

$$\sigma = \frac{\beta_0 - \alpha\left(1 + \frac{k_P}{k_{-B}}\frac{K_M^{H_2}}{[H_2]}\right)}{1 + \frac{K_I^{O_2}}{[O_2]} + \frac{k_B}{k_{-B}} + \frac{k_A}{k_{-A}}} \quad [15]$$

As equations 12 and 15 are both expressions for σ, they must be equal to one another, and so we write

$$\frac{\beta_0 - \alpha\left(1 + \frac{K_M^{H_2}}{[H_2]} + \frac{k_P}{k_{-B}}\frac{K_M^{H_2}}{[H_2]}\right)}{1 + \frac{k_B}{k_{-B}} + \frac{k_A}{k_{-A}}} = \frac{\beta_0 - \alpha\left(1 + \frac{k_P}{k_{-B}}\frac{K_M^{H_2}}{[H_2]}\right)}{1 + \frac{K_I^{O_2}}{[O_2]} + \frac{k_B}{k_{-B}} + \frac{k_A}{k_{-A}}} \quad [16]$$

Solving for α yields

$$\alpha = \frac{\beta_0 \frac{K_I^{O_2}}{[O_2]}}{\frac{K_M^{H_2}}{[H_2]}\left(1 + \frac{K_I^{O_2}}{[O_2]} + \frac{k_B}{k_{-B}} + \frac{k_A}{k_{-A}}\right) + \frac{K_I^{O_2}}{[O_2]}\left(1 + \frac{K_M^{H_2}}{[H_2]}\frac{k_P}{k_{-B}}\right)} \quad [17]$$

The overall rate of reaction, *v*, is defined as

$$v = k_{\text{cat}}\alpha \qquad [18]$$

and the maximum rate of reaction is obtained when $\alpha = \beta_0$

$$v_{\max} = k_{\text{cat}}\beta_0 \qquad [19]$$

The relationship between the rate of catalysis and the catalytic current, i, is given by

$$i = nFA\Gamma k_{cat} \qquad [20]$$

and we thus arrive at the final equation:

$$\frac{i}{i_{\max}} = \frac{\dfrac{K_{\text{I}}^{\text{O}_2}}{[\text{O}_2]}}{\dfrac{K_{\text{M}}^{\text{H}_2}}{[\text{H}_2]}\left(1+\dfrac{K_{\text{I}}^{\text{O}_2}}{[\text{O}_2]}+\dfrac{k_{\text{B}}}{k_{-\text{B}}}+\dfrac{k_{\text{A}}}{k_{-\text{A}}}\right)+\dfrac{K_{\text{I}}^{\text{O}_2}}{[\text{O}_2]}\left(1+\dfrac{K_{\text{M}}^{\text{H}_2}}{[\text{H}_2]}\dfrac{k_{\text{P}}}{k_{-\text{B}}}\right)} \qquad [21]$$