Electronic Supplementary Material (ESI) for Chemical Communications

The mechanism of inhibition by H_2 of H_2-evolution by hydrogenases
Supplementary information

1 Methods

Abbreviations

CHES: 2-[N-cyclohexylamino]ethenesulfonic acid.
DTT: dithiothreitol.
EDTA: 2-[2-(bis(carboxymethyl)amino)ethyl-(carboxymethyl)amino]acetic acid (ethylene diamine tetraacetic acid).
HEPES: N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid].
MES: 2-[N-morpholino] ethanesulfonic acid.
MV: methyl viologen (caution: toxic and suspected mutagen).
PGE: Pyrolytic graphite edge.
TAPS: N-tris[hydroxymethyl]methyl-3-aminopropanesulfonic acid.

1.1 Electrochemical methods

All PFV experiments were carried out in a glove box (Jacomex) under a N_2 atmosphere (O_2 < 1 ppm). The electrochemical cell was thermostated at the desired T using a water circulation system. A pyrolytic graphite edge (PGE) rotating disk working electrode (area $A \approx 3$ mm^2) was used in conjunction with an EG&G M636 electrode rotator, a platinum wire was used as a counter electrode, and a saturated calomel electrode (SCE), located in a Luggin side arm containing 0.1 M NaCl and maintained at room temperature, was used as a reference. All potentials are quoted versus the standard hydrogen electrode (SHE), $E_{SHE} = E_{SCE} + 241$ mV at room temperature. Experiments were performed with an Autolab electrochemical analyzer (PGSTAT 12, Eco Chemie).

The "mixed buffers" consisted of MES, HEPES, sodium acetate, TAPS, and CHES (5mM of each component), 1mM EDTA, and 0.1M NaCl as supporting electrolyte, titrated to the desired pH using concentrated HCl or NaOH.

Before preparing an enzyme film, the PGE electrode was polished with an aqueous alumina slurry (Buehler, 1 μm) and sonicated thoroughly. Protein films were prepared by painting the electrode with about half a microliter of a stock solution of enzyme (≈ 0.4mg/mL of NiFe hydrogenase in the mixed buffer at pH 7, or ≈0.07 mg/mL of clostridial FeFe hydrogenase in the purification buffer, consisting of 100mM TRIS HCl pH8, 150 mM NaCl, 2mM dithionite, 0.02 mM MV, 2.5mM destiobiotine), or ≈0.2 mg/mL of *Chlamydomonas reinhardtii* FeFe hydrogenase in a purification buffer, consisting of 100 mM Tris HCl, 150 mM NaCl, pH 8.3, 2 mM dithiothreitol, 2 mM Na-dithionite and 2.5 mM desthiobiotin.

To activate the NiFe enzymes, the enzyme-coated electrode was inserted in the electrochemical cell containing the mixed buffer at pH 4, 40°C, under an atmosphere of H_2, and poised at −560mV *vs* SHE for about one hour. The extent of activation was monitored by taking the electrode potential to −160mV to measure the H_2 oxidation current. The electrode could then be rinsed and transferred to a fresh solution with very little loss in electroactive coverage over time.

The concentration of H_2 in the cell solution was changed by directly bubbling either Ar (Linde, grade 4.5), Nidron 10 (called RH5 in the caption of fig 2), or H_2. The latter was produced using a 110H-MD Parker hydrogen gas generator (http://www.parker.com/dhi).

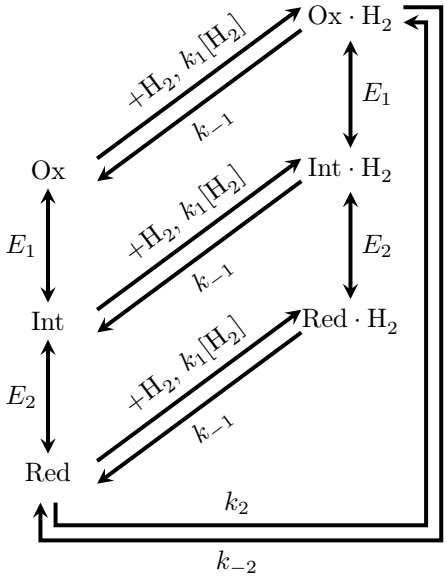
We analyzed and fitted the data using in-house programs called SOAS[1] and QSoas. The former is available free and free of charge on our Web site at http://bip.cnrs-mrs.fr/bip06/software.html. It is being replaced by an entirely new, powerful, open source program called QSoas, which will become available soon. Both programs embed the ODRPACK software for non-linear least squares regressions[2].

1.2 Preparation of the hydrogenase samples

Samples of WT *C. acetobutylicum* were prepared as described in refs 3,4. Samples of *C. reinhardtii* FeFe hydrogenases were prepared as described in refs 5. *Desulfovibrio hydrogenase* enzymes (WT and mutants) were purified as described previously[6].

2 Demonstration of equation (3)

We used a model similar to the one we developped earlier[7] in which H_2 diffuses inside the channel to reach a position in the vicinity of the active site termed the "geminate state". We assume that the presence of H_2 at this position and the redox chemistry at the active site are independent. Using a simple EEC model for the chemistry at the active site gives the following scheme:



Diffusion from and to the active site proceeds at rates $k_1[H_2]$ and k_{-1} respectively. Production of H_2 from the Red state proceeds at rate k_2, while the reverse reaction proceeds with rate k_{-2}. We name $E \cdot H_2$ (resp. E) the states of the enzyme with (resp. without) H_2 in the geminate state, i.e:

$$[E] = [Ox] + [Int] + [Red] \qquad [E \cdot H_2] = [Ox \cdot H_2] + [Int \cdot H_2] + [Red \cdot H_2] \tag{1}$$

Assuming that redox transitions are at equilibrium gives the following overall rate of production of H_2:

$$v_{H_2} = \frac{k_1 k_2^{\text{eff}} - k_1[H_2]k_{-2}^{\text{eff}}}{k_1[H_2] + k_2^{\text{eff}} + k_{-1} + k_{-2}^{\text{eff}}} \tag{2}$$

with:

$$k_2^{\text{eff}} = \frac{k_2}{1 + e_2 + e_1\, e_2} \qquad k_{-2}^{\text{eff}} = \frac{k_{-2}\, e_1\, e_2}{1 + e_2 + e_1\, e_2} \tag{3}$$

$$e_1 = \exp \frac{F\,(E - E_1)}{RT} \qquad e_2 = \exp \frac{F\,(E - E_2)}{RT} \tag{4}$$

Taking the low- and high-potential limits yields:

$$v_{H_2}^{\text{ox}} = -\frac{k_O}{1 + \frac{K_m}{[H_2]}} \qquad v_{H_2}^{\text{red}} = \frac{k_R}{1 + \frac{[H_2]}{K_i}} \tag{5}$$

with

$$k_R = \frac{k_{-1}\, k_2}{k_2 + k_{-1}} \qquad k_O = k_{-2} \qquad K_m = \frac{k_{-1} + k_{-2}}{k_1} \qquad K_i = \frac{k_{-1} + k_2}{k_1} \tag{6}$$

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