Electronic Supplementary Material (ESI) for Energy & Environmental Science

FeFe hydrogenase reductive inactivation and implication for catalysis

Abbreviations

CHES: 2-[N-cyclohexylamino]ethenesulfonic acid.
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.
PGE: Pyrolytic graphite edge.
TAPS: N-tris[hydroxymethyl]methyl-3-aminopropanesulfonic acid.

S1 Methods

Samples of *C. reinhardtii* FeFe hydrogenases were prepared as described in refs 1.

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 $A \approx 1.8$ 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 grafting the enzyme from Cr as fully described in ref 2.

The cell solution were saturated with H_2 by directly bubbling the gas 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[3] 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[4].

S2 Analysis of the data in main text fig 4

To analyse the data in fig 4, we assumed that each CV is the sum of two contributions, from the fully active and less active forms of the enzymes, which are the same for all CVs but their proportions vary as a function of time.

The CV obtained after full reactivation (purple in main text fig 4) was used as a reference to define the "fully active form", using the EEC model in ref 5 and adjusting the values of 4 independent parameters (we used an open-circuit potential of −438 mV vs SCE, measured during the experiment, to deduce the value of k_2/k_{-2} using the relation $E_{OCP} = \frac{E_1^0+E_2^0}{2} + \frac{1}{2f} \ln \frac{k_2}{k_{-2}}$).

The 26 CVs in fig 4B could then be modelled by adjusting a single set of the 4 parameters that define the curernt response of the "less active form" and 26×2 parameters that define the magnitudes of the two contributions (these 52 values are shown in main text fig 4D). The model was fitted by simultaneously analysing the CVs and the 1st derivatives of the CVs, as explained in ref 5.

The 8 parameters that define the two current/potential responses are listed in table S1.

As an example, figure S1 shows the fit of the 1st (blue) and last (purple) CVs shown in main text fig 4C. In the left panels, the blue and turquoise lines are the contributions of the two forms of the enzyme (fully active and less active, respectively).

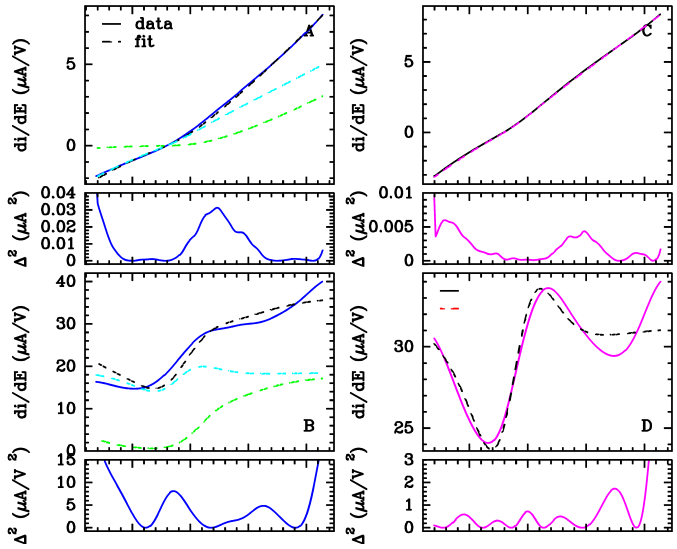

Figure S1: The simultaenous fit of the EEC model to the CVs (top) and 1st derivatives of the CVs (bottom) shown in main text fig 4, using the same color code.

	Fully active form (turquoise)		less active form (green)	
E_1^0 (mV *vs* SHE)	-405	± 1	-380	± 3
E_2^0 (mV *vs* SHE)	-472	± 1	-1000	± 400
k_2^0/k_1^0	0.05	± 0.04	2×10^{-3}	± 0.04
k_2/k^0	0.05	± 0.04	300	± 300

Table S1: Parameters that characterize the two forms of the enzyme, considering the model and the notations in ref 5.

S3 Determination of the characteristics of the "slow" and "fast" phases in figure 2

Reliable determination of the parameters of the exponential phases on figure 2 is delicate. The time dependence of the current on the low potential step can be described by

$$i(t) = \{i_\infty + \Delta i_{\text{fast}} \times \exp\{-(t-t_0)/\tau_{\text{fast}}\} + \Delta i_{\text{slow}} \times \exp\{-(t-t_0)/\tau_{\text{slow}}\}\} \times \exp\{-k_{\text{irrev}}(t-t_0)\} \quad \text{(S1)}$$

where Δi_{fast} is the absolute amplitude of the fast phase, τ_{fast} its time constant (and similary Δi_{slow} and τ_{slow} for the slow phase), k_{irrev} is the irreversible inactivation rate constant, i_∞ is the current that would be obtained at infinite time if there were no irreversible inactivation, and t_0 the time of the beginning of the step.

However, simply fitting equation (S1) to the low potential step is not satisfactory: the values of τ_{slow} and k_{irrev} are ill-defined, leading to indermination in the value of Δi_{slow}.

We therefore chose to constrain the value of the irreversible inactivation rate constant k_{irrev}. For that, we fit the three steps at once using equation (S1) for the low potential step, equation (S2) for the first step at high potential and equation (S3) for the second.

$$i(t) = i_{\text{hp}} \quad \text{(S2)}$$

$$i(t) = i_{\text{hp}} \times \exp\{-k_{\text{irrev}}\Delta t\} \times \{1 - \alpha \exp\{-(t-t_1)/\tau_{\text{react}}\}\} \times \exp\{-k'_{\text{irrev}}(t-t_1)\} \quad \text{(S3)}$$

i_{hp} is the initial current at high potential, $\Delta t = t_1 - t_0$ the time spent in the low-potential step, α the relative amplitude of reactivation and high potential, τ_{react} its time constant and k'_{irrev} the rate of the irreversible loss in the high potential step.

This method leads to a much better determination of the value of k_{irrev}, because it is imposed by the ratio of the current in the first step at high potential and that at the end of the second step at high potential. As a consequence, τ_{slow} and Δi_{slow} are also much better defined.

The relative amplitude of the fast and slow phases of inactivation is then given by:

$$\alpha_{\text{fast}} = \frac{\Delta i_{\text{fast}}}{i_\infty + \Delta i_{\text{fast}} + \Delta i_{\text{slow}}} \qquad \alpha_{\text{slow}} = \frac{\Delta i_{\text{slow}}}{i_\infty + \Delta i_{\text{fast}} + \Delta i_{\text{slow}}} \quad \text{(S4)}$$

References

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