

Simplicity in complexity: the photosynthetic reaction center performs as a simple 0.2 V battery

Bart J. van Rotterdam^{a,1}, Wim Crielaard^a, Ivo H.M. van Stokkum^c, Klaas J. Hellingwerf^a,
Hans V. Westerhoff^{a,b,*}

^aSwammerdam Institute for Life Sciences, BioCentrum Amsterdam, University of Amsterdam, Nieuwe Achtergracht 127, NL-1018 WS Amsterdam, The Netherlands

^bDepartment of Molecular Cell Physiology, Faculty of Biology, BioCentrum Amsterdam, Vrije Universiteit, de Boelelaan 1087, NL-1081 HV Amsterdam, The Netherlands

^cDepartment of Physics, Applied Computer Science, Faculty of Sciences, Vrije Universiteit, de Boelelaan 1081, NL-1081 HV Amsterdam, The Netherlands

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Abstract The photosynthetic reaction center is one of the most complicated molecular complexes. Transducing photon energy to a transmembrane electrochemical potential difference for protons, it is the direct or indirect energy source for virtually all life. We show here that it operates in a simple, battery-like manner, with a maximum potential of 0.20 V. Intriguingly this is only one fifth of the energy of the absorbed photon. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Photosynthesis; Proton motive force; Membrane potential; Reaction center; Non-equilibrium thermodynamics; Liposomes

Much of the energy used by living organisms to do work ultimately derives from photon energy harvested as electric energy. The photosynthetic reaction centers (PSRC) of photosynthetic bacteria, algae and green plants couple photochemistry to the translocation of an electron across the membrane dielectric in which they are embedded. Through the involvement of quinones, this results in a net proton translocation and in an electrochemical potential difference for protons across the membrane ($\Delta\tilde{\mu}_{H^+}$), which consists of an electric potential and a transmembrane pH difference. $\Delta\tilde{\mu}_{H^+}$ drives protons back across the membrane, in part through the H^+ -ATPase, which drives the synthesis of ATP [1–4].

Because the PSRC consists of a number of macromolecules and its activity spans various time domains its overall kinetic and thermodynamic properties might be expected to be highly complex and different from those of man-made electric batteries. The current delivered by the latter diminishes with increasing electric potential. A '1.5 V battery' cannot generate more than a 1.5 V potential, which is its electron motive force \mathcal{E}_e^0 . Only when both its internal resistance (R_i , Fig. 1A) and its electron motive force are very high, the battery becomes an ideal current source, delivering the same current independent of the counteracting potential.

Fig. 1 describes the correspondences between a light-driven proton pump and a battery. Electron flux between the two plates of a capacitor becomes proton flux across a membrane, the voltage difference becomes the proton electrochemical potential difference, $\Delta\tilde{\mu}_{H^+}$. The chemical (redox) reaction energizing the battery is the analogue of photon absorption, the external resistance the inverse of the proton conductance of the membrane. For the analogy to hold, the proton pumping rate of the PSRC should decrease with increasing $\Delta\tilde{\mu}_{H^+}$. Accordingly, there should be a maximum to the $\Delta\tilde{\mu}_{H^+}$ the PSRC can attain, which we define as $\mathcal{E}_{H^+}^0$.

To examine this we isolated PSRCs from *Rhodobacter sphaeroides* and reconstituted them into small unilamellar liposomes in the presence of UQ₀ (a water- and lipid-soluble ubiquinone analogue) and cytochrome *c*. This system functions as a light-driven proton pump (Fig. 1B,C) [5,6]. The transmembrane electric potential difference ($\Delta\psi$) was deduced from the changes in medium concentration of the lipophilic cation TPP⁺ [7,8]. Because the K⁺/H⁺ exchanger nigericin was added, $\Delta\psi$ could be equated to the $\Delta\tilde{\mu}_{H^+}$ [9]. The leak resistance for transmembrane proton movement (R_l) was modulated by the protonophore S-13. Fig. 2 plots the proton flux, derived as is described in Appendix 1, versus the transmembrane electric potential, for three different light intensities.

This current–voltage relationship of the PSRC turned out to be simply that of an electric battery, the delivered steady state current decreased with the counteracting electric potential. This was a surprise because boson-driven systems have been expected to be beyond comparison with other thermodynamic energy converters and to behave as ideal current sources [10], see however [11]. Extrapolating the current–voltage relationships to zero current determines the maximum potential that can be generated by the PSRC, $\mathcal{E}_{H^+}^0$ (Appendix 1). To determine this maximum potential we fitted straight lines through the data points and obtained a magnitude of approximately 0.20 V (Appendix 1), i.e. some 20 kJ/mol protons (Fig. 2).

One might expect the light intensity to affect the thermodynamic potential of the photons [12,13] and hence their effective driving force, $\mathcal{E}_{H^+}^0$. If so, then the abscissa intersection in Fig. 2 should increase with increasing light intensity. On the other hand, increasing the intensity of the incident light might

*Corresponding author. Fax: (31)-20-4447229.
E-mail address: hw@bio.vu.nl (H.V. Westerhoff).

¹ Present address: Molecular Biophysics, Debye Institute, Utrecht University, P.O. Box 80000, NL-3508 TA Utrecht, The Netherlands.

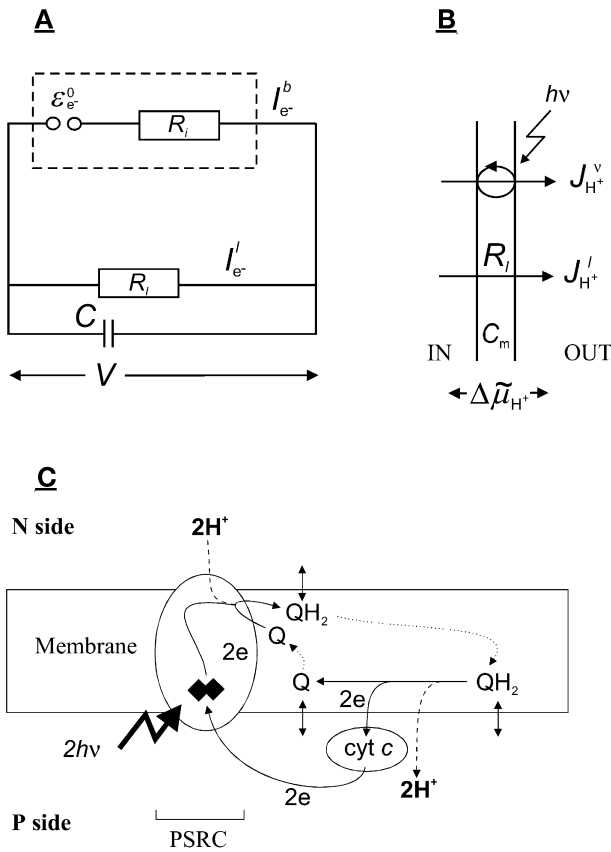


Fig. 1. Schemes comparing a man-made battery to the photosynthetic reaction center. A: Standard electric scheme for a battery: a voltage source generating ϵ_e^0 V and an internal resistance R_i . It delivers an electron current (I_e^b) to a capacitor (C) in parallel to an external resistance R_l . An electric potential (V) is generated across the capacitance. At steady state an equal current I_e^l flows through the load resistor R_l . B: The proton circuit of the photosynthetic reaction center/quinone system functioning as a light-driven proton pump. In a biological membrane, the PSRC absorbs a photon (as indicated by $h\nu$) and generates a proton flux (indicated as $J_{H^+}^v$). This flux charges the membrane capacitance (C_m) which leads to a transmembrane electrochemical potential difference for protons $\Delta\tilde{\mu}_{H^+}$. At steady state, a quantitatively equal proton flux $J_{H^+}^l$ flows back across the membrane, through a load such as the ATP synthesizing enzyme, or through leaks, represented here by a resistor to proton current R_l . C: The components in the reconstituted membrane that, together, transform the light-driven electron pump of the PSRC into a light-driven proton pumping system. cyt *c* represents cytochrome *c*, Q represents ubiquinone-0, QH₂ the doubly reduced, doubly protonated form thereof. The LH1 antenna complexes are omitted for clarity.

merely increase the frequency at which the PSRC is induced to function [14]. Then increasing the light intensity should not affect the abscissa intercept, i.e. not affect $\epsilon_{H^+}^0$. Fig. 2 suggests that the latter option is closest to the experimental reality. Indeed, three straight lines through a common intersection point at the abscissa fit the data well (Fig. 2 and Appendix 1). We conclude that the PSRC functions as if it were a 0.20 V man-made battery, with an internal resistance decreasing with light intensity (Appendix 1).

The observation that the maximum voltage is of macroscopic magnitude is not unexpected as the biological function of the PSRC is to generate a $\Delta\tilde{\mu}_{H^+}$ of some 20 kJ/mol, which corresponds to 0.20 V. This is small when compared to both the energy of the 870 nm photon it absorbs, i.e. 137 kJ/mol

photons and to the free energy (i.e. potential to do work) contained in the photons, i.e. 90 kJ/mol photons [12,15]. The PSRC appears to throw away more than 77% of the photon free energy.

Why is the PSRC not more efficient? Perhaps increasing its electron motive force above 0.2 V is dangerous, as 0.3 V exceeds the breakdown voltage of biological membranes [16]. 0.2 V is quite comparable to the voltages generated by the $\Delta\tilde{\mu}_{H^+}$ generators that are not driven by light, e.g. the respiratory chains of our mitochondria and of many bacteria [17]. Where does the missing free energy go? Free energy is dissipated when reactions run fast and reliably in a desired direction, or if they slip [14,18,19].

Biological systems that harvest free energy from light are of major interest for biotechnology. They may serve as prototypes for gadgets harvesting electric free energy directly from solar illumination. Incorporated in living systems, they may self-amplify and colonize niches where dead mechanical devices suffer from fouling. Our results therefore call for a further study of the mechanism by which the PSRC operates and of its potential to generate a higher electron motive force, or to pump more than one electron per photon [20]. A recent system reporting light-driven ATP production in membranes containing artificial PSRCs and natural H^+ -ATPase has not yet been analyzed in this respect [21,22]. Likewise it should be of interest to analyze the implications for steady state function, of kinetic studies of transmembrane electric potential effects on the PSRC [23–25].

Understanding the functioning of a living cell may seem tantalizing in view of the large number of its components. This problem is aggravated by the phenomenon of biocomplexity [26], i.e. that cellular components exhibit properties that transcend the properties they exhibit in isolation, in a way that is essential for biological function. The PSRC consists of molecules that are very large in size. Its functioning is different in the cell and in isolation, as it depends on the transmembrane electric potential. Scientists have shied away from such biocomplex systems, or have limited themselves to reporting phenomenological behavior without reference to molecular mechanism; the kinetic behavior of biocomplex systems was considered too complicated to be tractable. Our analysis here leads to a more optimistic view: yes, the PSRC is a biocomplex system, as it responds to the transmembrane electric potential. No, it is not intractable: its thermodynamic and kinetic behavior is quite well described in terms of a linear current–voltage relationship with a simple dependence on light intensity. The PSRC appears to be an example of simple biocomplexity, i.e. complex enough to be interesting and simple enough to make the physiological behavior accessible for detailed analysis.

Appendix 1. Calculations

The proton current versus proton electrochemical potential relationship of the photosynthetic reaction center may be described as follows:

$$J_{H^+}^v = \frac{n}{R_i^v} (\epsilon_{H^+}^0 - \Delta\tilde{\mu}_{H^+})$$

where $J_{H^+}^v$ equals the light-driven proton flux and n/R_i^v the proton to photon stoichiometry divided by the internal resis-

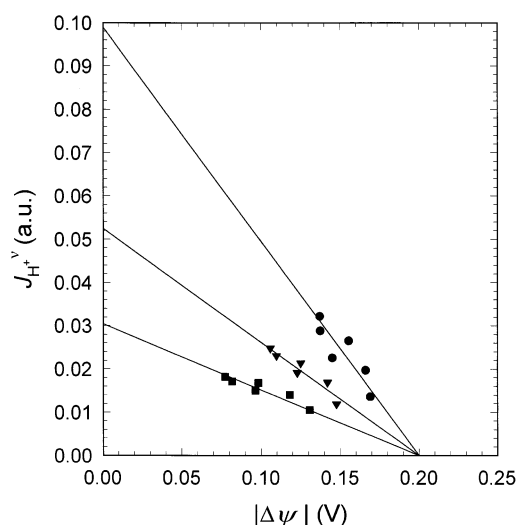


Fig. 2. Current–voltage relationship of the photosynthetic reaction center. The outward proton flux, $J_{H^+}^V$ is plotted versus $\Delta\Psi$ [9] which can be equated to the $\Delta\tilde{\mu}_{H^+}$ [9], for three different light intensities (box 1). $J_{H^+}^V$ is given in relative units as it is calculated by multiplying the proton motive force given in V times the membrane conductance given in the molar concentration of S-13 [6,15]. The solid lines are an orthogonal least squares fit based on our proposed model description for the PSRC experimental data (box 1). Key: squares, light intensity 1, 0.27 kW/m²; triangles, light intensity 2, 0.69 kW/m²; circles, light intensity 3, 2.81 kW/m².

tance of the reaction center. This equation is analogous to the current–voltage relationship of a man-made battery. See also Fig. 1A.

At each light intensity the data were modelled as: $J_{H^+}^V = (\epsilon_{H^+}^0 - \Delta\tilde{\mu}_{H^+})/R_i$ with parameters $\epsilon_{H^+}^0$ and R_i (the internal resistance). Both $J_{H^+}^V$ and $\Delta\tilde{\mu}_{H^+}$ contain measurement errors. We therefore applied an orthogonal least squares fit, weighting each data point by the reciprocal of the square root of its variance: $\text{var}(J_{H^+}^V) + \text{var}(\Delta\tilde{\mu}_{H^+})/R_i^2$ [27]. After fitting straight lines at each light intensity separately, we also fitted the data with a single $\epsilon_{H^+}^0$ parameter for all light intensities. These two models are nested, the former being the full model and the latter being the partial model, and thus can directly be compared [28]. The partial model resulted in a 5% decrease of the weighted root mean square error. A likelihood ratio test was performed using the calculated F -ratio of 0.26 with 2 and 12 degrees of freedom. This F -ratio is much smaller than $F(2,12,0.95) = 0.59$ and we therefore accept the partial model, which uses a single $\epsilon_{H^+}^0$ parameter for all light intensities. The parameter estimates and their standard deviations are: $\epsilon_{H^+}^0 = 0.20 \pm 0.01$, $R_{i,1} = 6.6 \pm 0.6$, $R_{i,2} = 3.8 \pm 0.4$ and $R_{i,3} = 2.0 \pm 0.3$, where the subscript indicates the light intensity, see legend to Fig. 2.

$J_{H^+}^V$ was derived using the measured transmembrane electric potential, $\Delta\Psi$, and the total conductance of the liposomal membrane, under the conditions at which the specific value for the $\Delta\Psi$ was determined. The load resistance, the inverse of the total conductance of the liposomal membrane, was calculated as follows. For any light intensity the inverse of the

transmembrane electric potential was plotted versus the concentration of added protonophore S-13. This was repeated for all three light intensities. The common intersection point of the three straight lines, calculated using a least squares fit (in a similar way as described earlier) was taken as $[S-13]_0$, the endogenous proton permeability of the membranes in units of [S-13] [9,15]. The resistance of the membrane to protons was equal to the inverse of $[S-13] + [S-13]_0$. At steady state the proton pumping flux should equal the proton leakage flux. According to Ohm's law the latter should be proportional to the transmembrane electric potential multiplied by $[S-13] + [S-13]_0$ (the overall proton conductance of the membrane), hence this product was taken as the pumped proton flux, $J_{H^+}^V$.

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