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Edited by

John F. Allen

Elisabeth Gantt

John H. Golbeck

Barry Osmond



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Kinetic Simulations of the C-Subunit of the Bacterial Reaction Center

Torsten Becker and G. Matthias Ullmann

Abstract Long range electron transfer in biological systems is known to depend on the protein environment and the charge state of neighboring redox-active cofactors. While the protein environment influences the equilibrium redox properties of the cofactors, their mutual interactions vary with time depending on the momentary charge state of all the cofactors. Continuum electrostatic calculations can be used to study the influence of the protein on the redox properties of the cofactors and to access the electrostatic interactions among them. The same theoretical framework can be used to simulate the kinetics of electron transfer, allowing one to account for the time-dependent interactions between cofactors during the transfer process. Here, we used this method to investigate the influence of the externally applied redox potential on the kinetic properties of electron transfer between the C-subunit and the special pair of the bacterial reaction center of *Blastochloris viridis*. Kinetic simulations of biological electron transfer are, thus, shown to provide a link between experimentally accessible external parameters such as the redox potential and the microscopic dynamics of the transfer system.

Keywords Electron transfer, Poisson-Boltzmann, kinetic simulation, photosynthetic reaction center, microstate

Introduction

The C-subunit, if present in the bacterial photosynthetic reaction center, facilitates electron transfer to the special pair via a chain of four heme cofactors (see Fig. 1). Three of the heme cofactors are histidine-methionine coordinated while one heme is histidine-histidine coordinated. The energetics of this transfer chain is often called a roller-coaster landscape, since it shows a peculiar arrangement of high-low-high-low potential hemes starting with the heme closest to the special pair.

The kinetics of this electron transfer system shows a strong dependence on the externally applied redox potential as shown by Ortega et al. (Ortega and Mathis 1993). The rereduction of the special pair is two times faster upon changing the applied redox potential from 380 to -20 mV. A recent study (Alric et al. 2006) pointed out the importance of accounting accurately for the interactions among those groups that actively participate in the transfer process.

Universität Bayreuth, Universitätsstrasse 30, 95447 Bayreuth

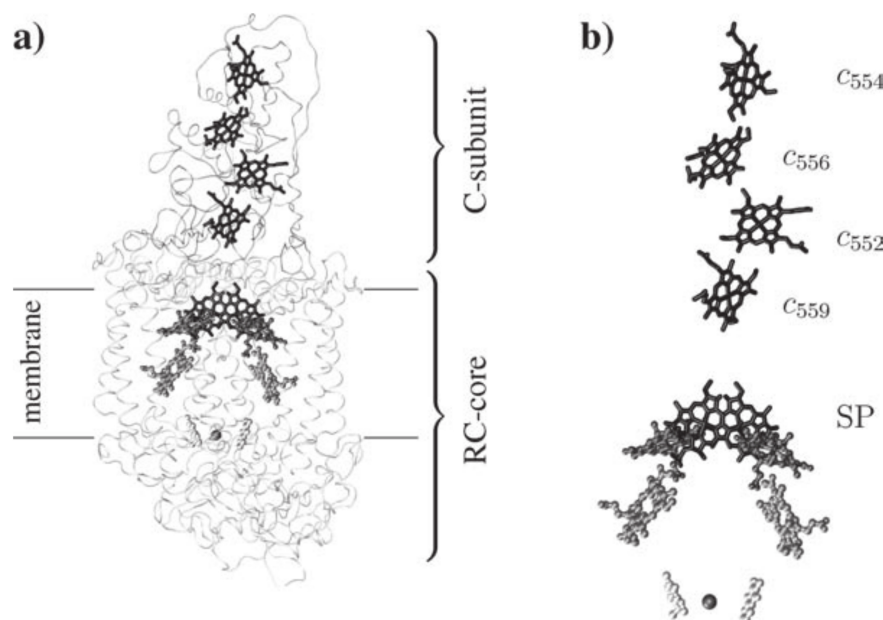


Fig. 1 (a) Reaction center with the C-Subunit of *Blastochloris viridis*. (b) Redox cofactors of the RC. The four hemes of the C-Subunit form a nearly linear transfer chain along the membrane normal. Electrons are transferred along this chain to reduce the special pair

In a previous work (Becker et al. 2007), we demonstrated that the interactions of redox active groups during electron transfer can be incorporated in kinetic simulations using a microstate description known from Poisson-Boltzmann electrostatic calculations. In order to relate experiment and simulations directly, the experimental settings have to be included in the simulations. Here, we study electron transfer in the C-subunit of the bacterial reaction center and its dependence on the externally applied redox potential.

Materials and methods

Microstate kinetics. Interactions between redox active cofactors can be taken into account by describing electron transfer in a microstate formalism (Becker et al. 2007). A microstate is described by a vector (x_1, \dots, x_N) , where x_i denotes the redox state of redox cofactor i . Electron transfer from cofactor i to cofactor j with respect to a given redox configuration of all other cofactors is then given as a transition from microstate $(x_1, \dots, 1_i, \dots, 0_j, \dots, x_N)$ to microstate $(x_1, \dots, 0_i, \dots, 1_j, \dots, x_N)$

where 1 and 0 denote a reduced and oxidized cofactor, respectively.

The energy of a microstate can be written as:

$$G(\vec{x}) = \sum_{i=1}^N (x_i - x_i^o) F (E_i^{\text{intr}} - E) + \frac{1}{2} \sum_{i=1}^N \sum_{j=1}^N (x_i - x_i^o) (x_j - x_j^o) W_{ij} \quad (1)$$

where x_i^o is the reference charge state, F the Faraday constant, E_i^{intr} denotes the intrinsic redox potential of cofactor i and W_{ij} accounts for the interaction of cofactors i and j . E is the externally applied redox potential.

Time evolution. We simulate the time evolution of electron transfer using a master equation:

$$\frac{d}{dt} P_v(t) = \sum_{\mu=1}^M k_{\mu v} P_{\mu}(t) - \sum_{\mu=1}^M k_{v\mu} P_v(t) \quad (2)$$

where P_v denotes the time dependent probability of microstate v and $k_{v\mu}$ is the transition probability from state μ to state v . It should be noted that the P_v are probabilities of microstates, not redox probabilities of individual cofactors.

The rate constants k_{vu} for exothermic transfer events were calculated according to the rate law formulated by Moser et al. 1992:

$$\log(k) = 13 - 0.6(R - 3.6) - 3.1 \frac{(\Delta G^\circ + \lambda)^2}{\lambda} \quad (3)$$

with R being the edge-to-edge distance of cofactors, ΔG° the energy difference between the involved microstates and λ being the reorganization energy.

The energy difference ΔG° was calculated from Eq. 1 using Poisson-Boltzmann electrostatics.

The Poisson-Boltzmann calculations were done using the MEAD package (Bashford and Gerwert 1992) using the structure of the reaction center of *Blastochloris viridis* (PDB code 1PRC; Higuchi et al. 1984).

The main component of the reorganization energy is also of electrostatic nature and can be calculated along the theory outlined by Sharp 1998. The remaining inner shell component of λ was calculated using the ADF density functional package (Guerra et al. 1998).

Details of the calculations can be found in (Becker et al. 2007).

Results and discussion

In this article we present simulations on the electron transfer kinetics of the C-subunit of the bacterial reaction center of *Blastochloris viridis* and its dependence on the externally applied redox potential.

With the help of Eq. 1 equilibrium distributions of microstates can be calculated for given redox potentials. Populations for cofactors can be calculated from the microstate distribution by summing up the respective contributions.

Within the range of -100 to 380 mV microstate populations were calculated in steps of 10 mV. These populations served as initial conditions for the kinetic simulations according to Eq. 2, where photooxidation was realized by setting the special pair to its oxidized state in the initial population of each simulation.

Figure 2 shows contour plots of the time dependent oxidation probabilities for the special pair and the

three highest potential heme cofactors. The cofactors are plotted in the order C556, C552, C559 and SPP. The lowest potential heme, C554, is nearly completely oxidized already at redox potentials around 0 mV and was thus omitted in Fig. 2.

The rereduction kinetics of the special pair (Fig. 2 SPP) shows three distinct regions. In the first region from -100 to ca. 50 mV the special pair becomes rereduced rapidly and completely. The half-life of the oxidized state in this region varies only slightly, increasing from 95 to 110 ns. Half lives have been obtained by fitting a single exponential to the time dependent oxidation probability. This corresponds well with the 100 ns measured by Ortega et al. at a redox potential of -20 mV.

A second region, from 50 to ca. 300 mV, again shows little variation in the rereduction kinetics of the special pair. A half life of ca. 200 ns is obtained for this second region, again in good agreement with the 190 ns measured at a redox potential of 250 mV.

Starting from 300 mV, the special pair is no longer fully rereduced. This gradual increase in oxidation of the special pair signifies the third region. The half life of the oxidized state, however, increases only slightly to 220 ns at 350 mV (taking into account, during the fitting procedure, that the special pair does not get fully reduced). Ortega et al. measured a half life of 220 ns for a redox potential of 380 mV, again in good agreement with our simulations.

Looking now at the three hemes (C556, C552 and C559), one can see that the three regions of the rereduction kinetics correspond to different cofactors serving subsequently as the electron reservoir according to their midpoint potentials. The first region corresponds to a oxidation of heme C552 (and heme C554; data not shown). The significant increase in the half-life of the special pair can be seen to correspond to the oxidation of heme C552. As it is known that the interaction between the cofactors C559 and C552 is responsible for the slowing down of the rereduction kinetics, our findings demonstrate that the microstate formalism proposed for kinetic simulations accurately includes these interactions.

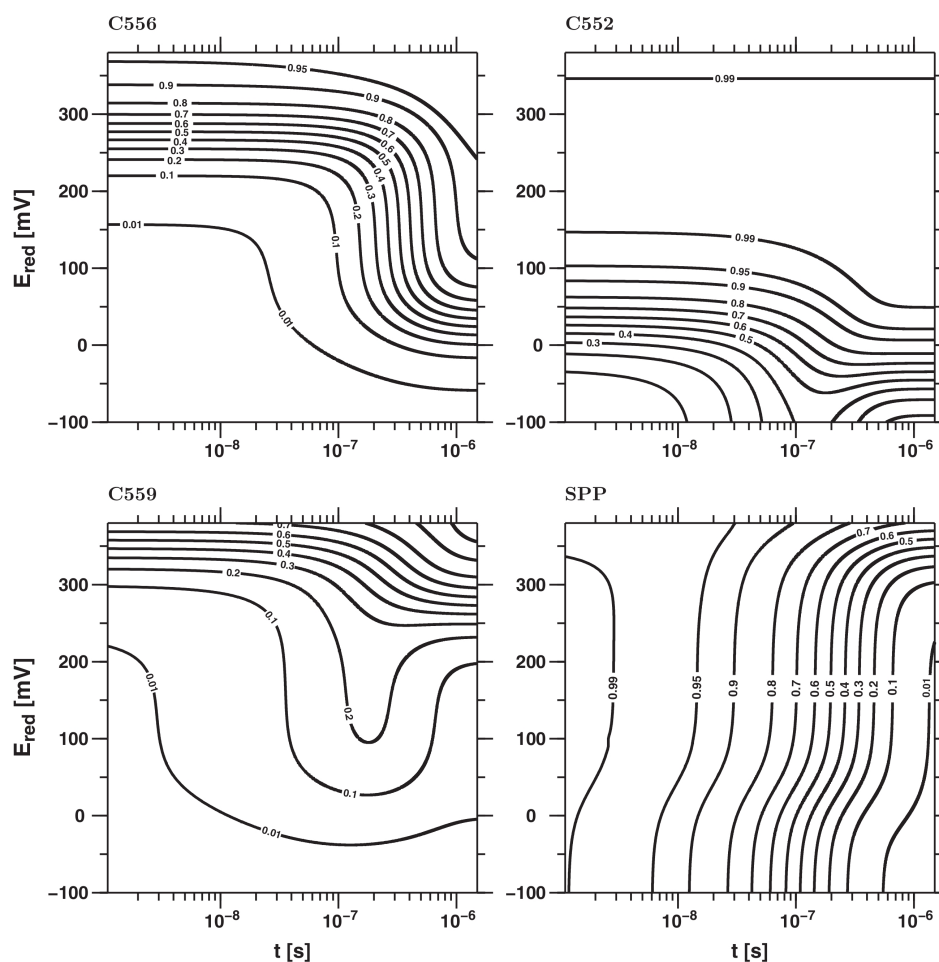


Fig. 2 Time dependence of the oxidation probability after photooxidation of the special pair for the heme cofactors C556, C552, C559 and the special pair, SPP. Plotted are lines of equal oxidation level

Finally, above 300 mV the initial reduction probability of the highest potential heme (Fig. 2 C559) decreases.

Thus, after photooxidation, the special pair does not become fully reduced. The kinetics of rereduction, however, are only slightly changed compared to region two. The two highest potential hemes interact only weakly as a consequence of their large edge-to-edge distance of 21 Å.

The descriptions given above are not evidence that the special pair is rereduced by different cofactors in these three regions. Indeed, it is known that the special pair always becomes rereduced via heme C559. Figure 2 relates the simulation results to those observed in an experimental study. Given agreement with experiment one can, however,

go one step further and analyze the underlying microstate dynamics. As shown in a previous study (Becker et al. 2007), the transfer dynamics strictly follow a next-neighbor rule, i.e., transfer events on the timescale of our simulations occur only between nearest neighbors. A direct consequence of these nearest neighbor interactions is the uphill step in energy that an electron has to take to go from heme C554 to heme C559.

Kinetic simulations of the type here proposed have the ability, due to their computational efficiency, to include experimentally controllable parameters such as the applied redox potential. It is, thus, possible to compare directly such simulations with experimental data in order help to analyze and interpret such data.

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