Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

Study of the Recombination Process of Light-Induced Charge Separation in Reaction Centers of Purple Bacteria Under Long-Term Exposition

Maryna Olenchuk [a] & Nataliya Berezetska [a]

[a] Institute of Physics, NAS of Ukraine, Kyiv, Ukraine

Version of record first published: 10 Jun 2010

To cite this article: Maryna Olenchuk & Nataliya Berezetska (2008): Study of the Recombination Process of Light-Induced Charge Separation in Reaction Centers of Purple Bacteria Under Long-Term Exposition, Molecular Crystals and Liquid Crystals, 497:1, 121/[453]-128/[460]

To link to this article: http://dx.doi.org/10.1080/15421400802458787

Mol. Cryst. Liq. Cryst., Vol. 497, pp. 121/[453]–128/[460], 2008

ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421400802458787

Taylor & Francis
Taylor & Francis Group

Study of the Recombination Process of Light-Induced Charge Separation in Reaction Centers of Purple Bacteria Under Long-Term Exposition

Maryna Olenchuk and Nataliya Berezetska
Institute of Physics, NAS of Ukraine, Kyiv, Ukraine

Experimental results, which confirm the nonlinear dynamic behavior of bacterial photosynthetic reaction centers under light-activated conditions, are presented. Different light-adapted conformational states of the reaction centers can be obtained by varying the exposition time. It is shown that the fast and slow equilibration kinetics of the reaction centers reflect the mechanisms of electron transfer processes. The reaction centers possessing light-induced structural changes, which have not relaxed completely before a flash, produce the pronounced slow relaxation component. This slow component splits into two separate components with increase in the exposition time. The amplitudes of the resulting components depend on the light adaptation time.

Keywords: charge-separated state; electron transfer; recombination kinetics

1. INTRODUCTION

Bacterial photosynthetic reaction centers are among the most comprehensively studied biological systems. The reaction centers from *Rhodobacter sphaeroides*, an anoxygenic purple nonsulfur bacterium, are well characterized both functionally and structurally [1]. A *Rb. sphaeroides* reaction center consists of three protein subunits (L, M, and H) and nine cofactors: a bacteriochlorophyll dimer (P composed of P_A and P_B), two accessory bacteriochlorophylls B_A and B_B, two bacteriopheophytins H_A and H_B, two quinones, Q_A and Q_B, and a non-heme iron (Fe). After the photooxidation of its primary donor (bacteriochlorophyll dimer), the RC switches to a long-living charge-separated state (the central reaction of photosynthesis), that is, to a state with the photomobilized electron transferred to the secondary

Address correspondence to Maryna Olenchuk, Institute of Physics, NAS of Ukraine, 46, Nauky Prosp., Kyiv 03028, Ukraine. E-mail: m.olenchuk@yahoo.com

quinone acceptor Q_B. The kinetics of these processes is reflected by the time-dependent absorbance changes of the RC optical marker (the band near 865 nm for RCs from *Rb. sphaeroides*).

Many researchers have discussed light-induced conformational transitions in RCs [2–4]. The charge recombination rate constant in RCs depends on structural coordinates such as the donor-acceptor distance [5,6]. These authors experimentally determined, in fact, the distribution function for a generalized conformational coordinate both in dark and under illumination by quenching the structural relaxation at cryogenic temperatures. They obtained a light-induced increase in the donor-acceptor distance by $\sim$1 Å. EPR studies of RCs showed that light-induced conformational changes are not simple relative translations of the donor and primary quinone acceptor (Q_A) molecules, but that they are more likely rearrangements of the protein structure [7]. More recent x-ray studies of RCs from *Rb. sphaeroides* showed a large ($\sim$5 Å) light-induced translation of the secondary quinone acceptor from its location in the dark-adapted system accompanied by a 180° rotation about the isoprene axis [8]. These authors also reported light-induced changes in the protein structure that affect the protonation of amino acid residues. Recent molecular dynamic calculations demonstrated the existence of two distinctly different binding sites for the neutral secondary quinone Q_B^- and semiquinone anion Q_B^- [9]. The authors showed for the first time that the protonation of ASP L213 should occur prior to the occupation by Q_B^- of its stable (quasi-equilibrium) site $\sim$5 Å distant from the site which is at equilibrium for a neutral quinone in dark. Such a motion of an ubisemiquinone Q_B^- from the nonequilibrium position that is characteristic of the dark-adapted structure to a quasiequilibrium position that is stable for the light-adapted structure indicates the importance of nonequilibrium structural transitions in RCs. These observations explain the previously reported light-induced changes in the transient absorption spectrum of *Rb. sphaeroides* RCs [5], but the physical phenomena responsible for these new conformations remained unexplained.

In the present work, we explore the kinetics of light-induced absorption changes in RCs. We present experimental results that confirm the existence of the protein structural memory that lasts for a time longer than the interval between consecutive turnovers of the reaction center—a necessary condition for the nonlinear self-organization mechanisms in the electron transfer system of an RC. The outline of this paper is the following: 1) we show that the lifetime of the charge-separated state reflects the light-induced structural changes in the system; 2) our study confirms that the slow structural dynamics

leads to self-regulation phenomena in biological macromolecules, which is in line with the previous theoretical developments [10,11].

2. METHODS AND MATERIALS

We used isolated RCs from photosynthetic bacteria *Rb. sphaeroides* (wild-type and the strain R26). These RCs were isolated and purified from the photosynthetic membranes according to the procedure described elsewhere [12].

An optical setup designed in our laboratory was used. The sample was placed in the solutions in a 1-mm pathlength quartz cuvette. The sample was photoexcited by four red light emitting diodes (LEDs) with the total power at the sample of $0.375\,\mathrm{mW/cm^2}$. The time-dependent absorbance changes of the RCs at 865 nm were measured at the prolonged photoexcitation. The illumination duration was from 1 to 50 s. The intensities of the probe beam and the background excitation were monitored simultaneously to account for possible instabilities in the light sources. The cuvette temperature was stabilized at $15 \pm 0.5^{\circ}\mathrm{C}$. Data collection and experimental control was provided by a PC with a plug-in data acquisition board. The details of the experimental setup can be found elsewhere [13,14].

3. RESULTS AND DISCUSSION

Figure 1 shows the experimental dependences of the absorbance changes at 865 nm with steady-state illumination intensity ($I = 0.375\,\mathrm{mW/cm^2}$). The curves correspond to different exposition times.

We assume that the system is closed and possesses a fixed number of localized electron states. These assumptions are supported by the following observations. The RCs undergo the complete electron relaxation during several minutes after the termination of a prolonged illumination. The total time of equilibration depends on the photoactivation time.

As clearly seen from Figure 1, the decreasing parts of all curves describe the charge recombination process measured upon turning the photoactivation off. These parts of the curves have two pronounced relaxation phases: fast and slow ones.

The absorption recovery kinetics determines main kinetic parameters of the electron transfer reactions in RCs. The conventional way of obtaining these parameters is to approximate the decay curves with a set of exponents. However, in the case of biological molecules, the influence of structural relaxations leads to extremely complex multiexponential kinetics [15–17]. As a result, the choice of the number of

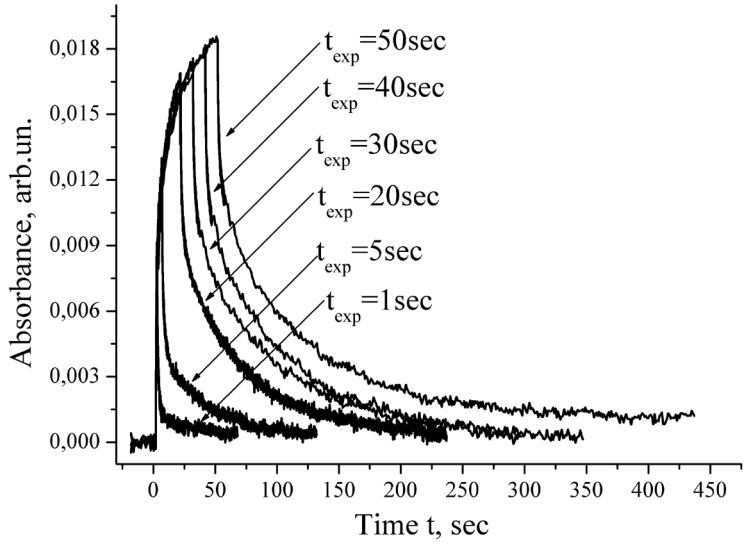

FIGURE 1 RC bleaching and recovery kinetics measured at the maximum of the primary donor absorption band at $\lambda_{max} = 865$ nm and after a stepwise increase of the exposition time.

exponential components becomes ambiguous. It is well known that the majority of multidimensional optimization problems with a large number of variables are usually ill-posed [18,19]. This means that there is an almost infinite number of possible solutions which provide the same accuracy in terms of the root-mean-square deviation from the given reference. Many different techniques were proposed to solve such ill-posed optimization problems. We used the Maximum Entropy Method (MEM) which is one of the most powerful and robust optimization techniques available to date [18,20] to analyze the continuous spectrum $\rho(\tau)$ of lifetimes τ of separated charges relative to the recombination according to the relation $\Delta A_{rel}(t) = \int \rho(\tau) e^{-t/\tau} d\tau$. Here, $\Delta A_{rel}(t)$ is the absorption recovery kinetics of RCs, τ is the average lifetime, $\rho(\tau)$ is the distribution of the lifetimes, and t is the time. The computer program is developed in our laboratory and available at http://www.geocities.com/memfit_group/index.htm. Our tests have shown that the MEM optimization technique allows one to solve the problem of choosing the number of components easily. MEM always has a solution with minimal information content and stable distribution of τ.

The distribution function of the survival time, $\rho(\tau)$, has peaks that correspond to some kinetic component. As is evident from Figure 2, there are two phases of a relaxation (fast and slow) of the RC recovery

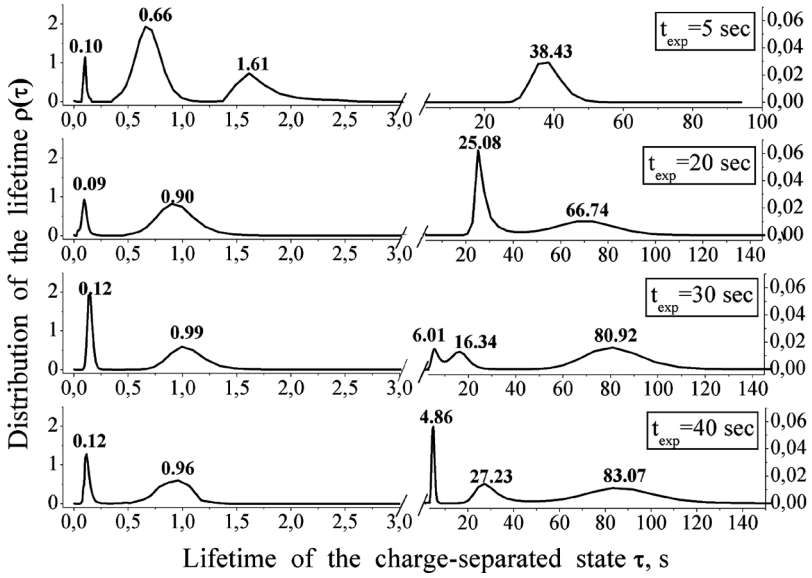

FIGURE 2 Distribution function of the lifetime $\rho(\tau)$ for various exposition times.

kinetics. The amplitudes of these phases are always comparable. The presence of the fast kinetic component with $\tau_1 \approx 0.1\,\text{s}$ corresponds to RCs with the inactive Q_B, i.e., those without the secondary acceptor [1,8]. The kinetic component with $\tau_2 < 1\,\text{s}$ corresponds to the fast electron recombination from the secondary quinone. The peak with $\tau_3 \approx 1.6\,\text{s}$ which was called a "satellite" peak is observed for small photoactivation times (up to $t_{\text{exp}} = 5\,\text{s}$). It disappears at longer exposition times.

The nature of the satellite peak corresponds to a redistribution of the cofactors' electronic state populations under excitation. For the RCs with a finite number of different charge-transfer states, the quasistationary populations of these states with a localized electron on different cofactors may change. Such population changes are caused by the light-induced structural changes [10,11]. The self-consistent statistical theory of the charge transfer and structural motions was proposed in [10,21]. In this theory, the structural dynamics of an RC and the electron transfer are considered in a self-consistent manner. This theory provides a description of the electron-conformational interaction in RCs by considering the diffusion of the system along the conformational coordinate in an effective adiabatic potential. This potential determines the average value

of x (a global configurational coordinate that describes slow photoinduced structural changes) over the electron distribution function. This potential is of a statistical nature and depends on the stationary-state distribution of localized electron populations at a fixed structure. This structure is determined and controlled by the illumination intensity I. The light-induced deformation of the conformational potential causes a change of the distribution of localized electron populations. The maxima of this distribution functions shift toward larger values of the conformational coordinate [10]. The dependence of this distribution on the light intensity was described previously in [6,10,22]. A change of the shape of this distribution influences the "satellite" peak behavior. The value of a maximum of the peak τ_2 becomes 1 s, while the peak τ_3 disappears, and the positions of the peaks $\tau_1 \approx 0.1$ s and $\tau_2 \approx 1$ s do not change at exposition times of 10 s and more.

The value of τ_4 may be compared with the characteristic time of a slow structural rearrangement in the RC. In this case, the recombination process may be viewed as an electron-conformational relaxation process. When the exposition time increases, a displacement of the peak τ_4 (slow kinetic component) is observed. The slow component corresponds to an increase of the energy of interaction of the electron with a place of localization of secondary quinone [8]. The lifetime of the slow kinetic component was $\tau_4 \approx 29s$ for the photoactivation time $t_{\text{exp}} = 1$ s (not shown). The longer exposition time ($t_{\text{exp}} = 5$ s) leads to a considerably slower relaxation with the lifetime $\tau_4 \approx 38.4$ s. Moreover, in the case where the RCs were exposed for tens of seconds, a new peak appears in the spectrum as a result of the splitting of the existing peak (Fig. 2). It is assigned to the photoconformational rearrangement induced by a relaxation of the structure in the charge-separated state. With increase in the exposition time ($t_{\text{exp}} = 30$ s),the redistribution between conformational components takes place. The number of "slow" kinetic components increases (Fig. 2). Values of the lifetimes of the slow kinetic component are $\tau_4^1 \approx 80.9$ s, $\tau_4^1 \approx 80.9$ s, and $\tau_4^3 \approx 6$ s for the exposition time $t_{\text{exp}} = 30$ s; $\tau_4^1 \approx 83.1$ s, $\tau_4^2 \approx 27.2$ s, and $\tau_4^3 \approx 4.9$ s for the exposition time $t_{\text{exp}} = 40$ s; and $\tau_4^1 \approx 88.3$ s, $\tau_4^2 \approx 42.2$ s, and $\tau_4^3 \approx 9.6$ s for the exposition time $t_{\text{exp}} = 50$ s. Large changes in the electron transfer and charge recombination kinetics of RCs upon the prolonged illumination are not related to the loss of the photochemical activity of RCs, but rather to the formation of new "light-induced" conformations of RCs [4]. The rate of recombination depends on the value of the structural variable and can vary continuously. The system moves along a chosen trajectory in the conformational space during the relaxation which is accompanied by a decrease of the free energy. At long times of

photoactivation, this trajectory splits at some point, which corresponds to the appearance of slow relaxation components τ_4^1, τ_4^2, and τ_4^3. The motion along each of the resulting trajectories is characterized by different recombination rates.

4. CONCLUSION

The experimental analysis of the recombination kinetics of photosynthetic RCs under a long time of photoactivation is presented. The existence of two relaxation phases implies that the recombination kinetics is determined by either an almost nondeformed (fast recombination phase) or heavily deformed (slow recombination phase) conformation of RCs. This reflects the bistable nature of the conformational potential. The "light-adapted" conformational state is observed in the system when a new minimum in the conformational potential appears [22]. In this state, the lifetime of the charge-separated state may be quite different from that of the "dark-adapted" state. It was shown that the slow relaxation component splits at long times of photoactivation (10 s and more). The values of the lifetime of slow components change differently. Such a dynamics testifies that the localization of electrons on quinone acceptors under long photoactivation times leads to conformational changes in the structure of RCs that can be accumulated over subsequent excitation events and preserved for a long period of time. This modification influences the efficiency of the electron tunneling and stimulates the transition from the "light-adapted" conformational state to the state with a qualitatively different shape of the effective conformational potential.

REFERENCES

[1] Hoff, A. J. & Deisenhofer, J. (1997). *Physics Reports*, *287*, 2.
[2] Graige, M. S., Feher, G., & Okamura, M. Y. (1998). *Proc. Natl. Acad. Sci. USA*, *95*, 11679.
[3] McMahon, B. H., Müller, J. D., Wraight, C. A., & Nienhaus, G. U. (1998). *Biophys. J.*, *74*, 2567.
[4] Kalman, L. & Maroti, P. (1997). *Biochemistry*, *36*, 15269.
[5] Kleinfeld, D., Okamura, M. Y., & Feher, G. (1984). *Biochemistry B*, *23*, 5780.
[6] Shaitan, K. V., Uporov, I. V., Lukashev, E. P., Kononenko, A. A., & Rubin, A. B. (1991). *Mol. Biol.*, *25*, 560.
[7] Zech, S. G., Bittl, R., Gardiner, A. T., & Lubitz, W. (1997). *Appl. Magn. Reson.*, *13*, 517.
[8] Stowell, M. H. B., McPhillips, T. M., Rees, D. C., Soltis, S. M., Abresch, E., & Feher, G. (1997). *Science*, *276*, 812.
[9] Grafton, A. K. & Wheeler, R. A. (1999). *J. Phys. Chem.*, *103*, 5380.
[10] Goushcha, A. O., Kharkyanen, V. N., Scott, G. W., & Holzwarth, A. R. (2000). *Biophys. J.*, *79*, 237.

[11] Christophorov, L., Holzwarth, A., Kharkyanen, V., & van Mourik, F. (2000). *Chem. Phys.*, *256*, 45.
[12] Zakharova, N. I., Fabion, M. Ya., Uspenskaya, N. Ya., Kononenko, A. A., & Rubin, A. B. (1981). *Biokhim.*, *46*, 1703.
[13] Mueller, M. G., Griebenow, K., & Holzwarth, A. R. (1991). *Biochim. Biophys. Acta*, *1098*, 1.
[14] Goushcha, A. O., Kharkyanen, V. N., & Holzwarth, A. R. (1997). *J. Phys. Chem. B.*, *101*, 259.
[15] Frauenfelder, H., Sligar, S. G., & Wolynes, P. G. (1991). *Science*, *254*, 1598.
[16] Frauenfelder, H., Wolynes, P. G., & Austin, R. H. (1999). *Rev. Mod. Phys.*, *71*, S419.
[17] Barabash, Yu. M., Berezetskaya, N. M., Christophorov, L. N., Goushcha, A. O., & Kharkyanen, V. N. (2002). *J. Chem. Phys.*, *116*, 4339.
[18] Skilling, J. (1989). Classic maximum entropy In: *Maximum Entropy and Bayesian Methods*, Skilling, J. (Ed.), Kluwer: Academic, Norwell, MA, 45–52.
[19] Steinbach, P. J., Chu, K., Frauenfelder, H., Johnson, J. B., Lamb, D. C., Nienhaus, G. U., Sauke, T. B., & Young, R. D. (1992). *Biophys. J.*, *61*, 235.
[20] Steinbach, P. G., Ionescu, R., & Matthews, C. R. (2002). *Biophys. J.*, *82*, 2244.
[21] Goushcha, A. O., Manzo, A. J., Scott, G. W., Christophorov, L. N., Knox, P. P., Barabash, Yu. M., Kapoustina, M. T., Berezetska, N. M., & Kharkyanen, V. N. (2003). *Biophys. J.*, *84*, 1146.
[22] Uporov, I. V. & Shaitan, K. V. (1990). *Khimich. Fiz.*, *9*, 992.