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Review

Probing light-induced conformational transitions in bacterial photosynthetic reaction centers embedded in trehalose–water amorphous matrices

Francesco Francia[a], Gerardo Palazzo[b,f], Antonia Mallardi[c], Lorenzo Cordone[d,e], Giovanni Venturoli[a,e,*]

[a] *Laboratorio di Biochimica e Biofisica, Dipartimento di Biologia, Università di Bologna, 40126 Bologna, Italy*
[b] *Dipartimento di Chimica, Università di Bari, 70126 Bari, Italy*
[c] *Istituto per i Processi Chimico-Fisici (IPCF), CNR, 70126 Bari, Italy*
[d] *Dipartimento di Scienze Fisiche ed Astronomiche, Università di Palermo, 90123 Palermo, Italy*
[e] *Istituto Nazionale per la Fisica della Materia (INFM), Italy*
[f] *Consorzio Interuniversitario per lo sviluppo dei Sistemi a Grande Interfase (CSGI), 50019 Sesto Eiorantino, Italy*

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Abstract

The coupling between electron transfer and protein dynamics has been studied in photosynthetic reaction centers (RC) from *Rhodobacter sphaeroides* by embedding the protein into room temperature solid trehalose–water matrices. Electron transfer kinetics from the primary quinone acceptor (Q_A^-) to the photoxidized donor (P^+) were measured as a function of the duration of photoexcitation from 20 ns (laser flash) to more than 1 min. Decreasing the water content of the matrix down to $\approx 5 \times 10^3$ water molecules per RC causes a reversible four-times acceleration of $P^+Q_A^-$ recombination after the laser pulse. By comparing the broadly distributed kinetics observed under these conditions with the ones measured in glycerol–water mixtures at cryogenic temperatures, we conclude that RC relaxation from the dark-adapted to the light-adapted state and thermal fluctuations among conformational substates are hindered in the room temperature matrix over the time scale of tens of milliseconds. When the duration of photoexcitation is increased from a few milliseconds to the second time scale, recombination kinetics of $P^+Q_A^-$ slows down progressively and becomes less distributed, indicating that even in the driest matrices, during continuous illumination, the RC is gaining a limited conformational freedom that results in partial stabilization of $P^+Q_A^-$. This behavior is consistent with a tight structural and dynamical coupling between the protein surface and the trehalose–water matrix.

Keywords: Photosynthetic reaction center; Trehalose; Electron transfer; Protein dynamics; Conformational relaxation

1. Introduction

Proteins are characterized by complex conformational dynamics. The wide range of internal motions they experience at physiological temperatures originates from rugged energy landscapes, which feature an extremely large number of minima corresponding to different conformational substates, organized in hierarchical tiers [1–5]. This ability of the protein to perform structural fluctuations among many different conformational substates appears to be intimately connected to protein function [6–8].

The photosynthetic reaction center (RC) from purple bacteria is becoming a paradigmatic system in the study of the relationship between electron transfer processes and protein conformational dynamics. This integral chromoprotein, following photon absorption by the primary electron donor P (a bacteriochlorophyll dimer), catalyzes a sequential electron tunneling, which leads in 200 ps to reduction of the primary quinone acceptor Q_A, located 25 Å away from P. From Q_A^-, the electron is then delivered to the secondary quinone acceptor Q_B. In the absence of electron donors to P^+, the electron on Q_B^- recombines with the hole on P^+. In Q_B-deprived RCs, recombination of the primary

* Corresponding author. Department of Biology, University of Bologna, Alma Mater Studorium, Via Irnerio 42, I-40126 Bologna, Italy. Tel.: +39-51-242576; fax: +39-51-251208.
E-mail address: ventur@alma.unibo.it (G. Venturoli).

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charge separated state $P^+Q_A^-$ takes place by direct electron tunneling [9].

A large number of experimental findings indicates that the electric field generated by light-induced charge separation within the RC perturbs substantially the protein giving rise to conformational changes, which in turn affect the electron transfer kinetics (see, e.g., Refs. [10–14]). A basic strategy to gain insight into the coupling between electron transfer and protein motions consists in modulating the rate of conformational relaxations and the interconversion among protein conformational substates by changing the temperature. Fundamental information was obtained by comparing the kinetics of electron transfer in RCs frozen at cryogenic temperatures in the dark and under illumination. These studies have unambiguously shown that RCs can be trapped at cryogenic temperatures in a *dark-adapted* and a *light-adapted* conformation, which drastically differ in the stability of the primary charge separated state $P^+Q_A^-$. Each conformation is characterized by a large distribution of substates, as inferred from the strongly distributed, nonexponential kinetics of $P^+Q_A^-$ recombination at cryogenic temperatures [11,14]. According to the dynamic model proposed by McMahon et al. [14], at physiological temperatures, the RC protein, following light-induced transition to the $P^+Q_A^-$ state, relaxes rapidly from the dark-adapted to the light-adapted conformation by solvating the altered charge distribution. This relaxation is accompanied by a decrease in the energy gap between $P^+Q_A^-$ and the neutral state PQ_A, which is reflected in a decrease of the electron transfer rate, i.e., in a stabilization of the charge-separated state. At room temperature, the RC protein rapidly samples the distribution of conformational substates and averaging of the corresponding rate distribution over the time scale of charge recombination gives rise to an almost exponential kinetics of $P^+Q_A^-$ recombination [14].

A second reaction, intensively studied in relation to RC dynamics, is electron transfer from Q_A^- to the secondary quinone acceptor Q_B. When RCs are frozen in the dark below 200 K, Q_A^--to-Q_B electron transfer is hindered; at variance, it persists even at 50 K if RCs are frozen under illumination [11, 15]. This electron transfer process, which appears to be governed by a complex energy landscape [16], has been proposed to be conformationally gated [17].

We have recently shown that incorporation of the RC into a dehydrated amorphous trehalose matrix severely restricts the RC conformational dynamics *at room temperature* as judged from the effects on the kinetics of specific electron transfer processes [18,19]. This approach, originally developed to analyze function-dynamics coupling in heme proteins, provides an attractive, complementary alternative to investigations in which the slowing down of the protein dynamics is obtained by lowering the temperature. In fact, in *trehalose-coated* RCs the coupling between RC dynamics and electron transfer can be examined at physiological temperatures, thus uncoupling, in principle, temperature from solvent effects.

The dynamic behavior of trehalose-coated soluble proteins has been extensively investigated [20–22] also in view of the peculiar efficacy of trehalose in the preservation of biostructures [23]. Both spectroscopic studies [24] and molecular dynamics simulations performed on myoglobin–trehalose–water systems [25,26] have shown that the residual water content modulates the protein motional freedom.

We have previously found that a progressive dehydration of RC–trehalose–water matrices first affects Q_A^--to-Q_B electron transfer: Upon drying, the reaction is arrested in a progressively increasing fraction of the RC population and complete inhibition is attained in relatively wet glasses [19]. This behavior is fully consistent with a conformational gate mechanism. When the amount of residual water in the amorphous matrix is further reduced, kinetic analysis of flash-induced $P^+Q_A^-$ recombination reveals that RC relaxation from the dark-adapted to the light-adapted conformation as well as interconversion between conformational substates are dramatically hindered [18]. This is inferred from the distributed and accelerated $P^+Q_A^-$ recombination kinetics, which, in the more dried matrices at room temperature, are close to those measured in glycerol–water mixtures at cryogenic temperature [14]. As a whole, the studies performed on trehalose-coated RCs suggest that the conformational dynamics controlling electron transfer is strongly and specifically slaved to the structure and dynamics of the surrounding medium.

In the present work, we analyze the kinetics of $P^+Q_A^-$ recombination following continuous illumination of RCs embedded into dry trehalose matrices. By this strategy, we further characterize the restricted RC dynamics, showing that, during continuous photoexcitation, a partial stabilization of $P^+Q_A^-$ recombination (reflecting slowed protein relaxation) is attained within approximately 1 s even in solid samples of extremely low content of residual water.

2. Materials and methods

The RCs were purified from *Rhodobacter sphaeroides* R-26 according to Gray et al. [27]. The secondary quinone Q_B was removed ($\approx$ 98%) following the procedure described in Ref. [28]. Trehalose (α-D-glucopyranosyl α-D-glucopyranoside) was from Sigma (>99% purity) and used without further purification. For sample preparation, RC were diluted to 40 μM in 5 mM Tris, pH 8.0, 0.4 M trehalose, 0.025 lauryl dimethylamine-N-oxide. This yields a trehalose to RC molar ratio equal to 10^4, i.e., a sugar to protein ratio lower than that ($\approx 1.5 \times 10^4$) used in our previous studies [18,19]. A thin layer of the above solution, deposited on an optical glass plate, was initially dried for $\approx$ 3 h under N_2 flow, obtaining a transparent plasticized sample. Maximal dehydration was achieved by leaving the matrix under dry N_2 atmosphere at 30 °C for several days. The water to RC molar ratio in dehydrated trehalose matrices was estimated

by near-infrared (NIR) spectroscopy on the basis of the combination band of water at 1950 nm and of the RC absorption band at 802 used as an internal standard [18]. $P^+Q_A^-$ recombination was monitored at 298 K with a spectrophotometer of local design [18] at 422, 450, 542, and 605 nm [29] obtaining essentially the same kinetics. RC were photoactivated by a 20-ns pulse from a dye-laser-cavity (RDP-1 Radiant Dyes GmbH, Wermelskirchen, Germany) pumped by a frequency-doubled Q-switched Nd-YAG laser (Surelite 10, Continuum, Santa Clara, CA), using stryryl 9 as a dye (λ_{max} at 810 nm). Continuous RC photoexcitation was accomplished by a collimated 200-W quartz tungsten halogen lamp as described in Ref. [19]. No temperature increase of the sample was detected during continuous illumination with a sensitivity of 0.1 °C. Samples were dark-adapted for at least 2 min between each measurement. Nonlinear least-square minimization and numerical determination of confidence intervals of fitting parameters were performed as detailed in Ref. [18].

3. Results

The photo-induced charge separation and the subsequent charge recombination in Q_B deprived RC is described by

$$PQ_A \underset{k}{\overset{h\nu}{\rightleftarrows}} P^+Q_A^- \tag{1}$$

Fig. 1 exemplifies the approach used in analyzing the recombination kinetics of the $P^+Q_A^-$ state formed by a laser pulse in RC incorporated into amorphous trehalose–water matrices. As evidenced in a previous systematic study performed in progressively dehydrated matrices [18], description of the kinetics, which become progressively non-exponential upon dehydration, requires a continuous rate distribution $p(k)$. The survival probability of the $P^+Q_A^-$ state is given by

$$N(t) = \frac{P^+Q_A^-(t)}{P^+Q_A^-(0)} = \int_0^\infty p(k)e^{-kt}dk \tag{2}$$

The rate distribution $p(k)$ is well approximated by a gamma distribution, i.e.,

$$p(k) = \frac{k^{n-1}}{k_0^n}\frac{\exp\left(-\frac{k}{k_0}\right)}{\Gamma(n)} \tag{3}$$

where $\Gamma(n)$ is the gamma function. Taking the Laplace transform of Eq. (3) yields on the time domain a simple description of the charge recombination kinetics, i.e.,

$$N(t) = \mathcal{L}[p(k)] = (1 + k_0 t)^{-n} \tag{4}$$

This power law, which has been previously used to describe $P^+Q_A^-$ recombination at cryogenic temperatures [11], fits well $N(t)$ in trehalose solutions and dehydrated amorphous matrices over the whole range of residual water content explored [18]. Parameters k_o and n are related to the average rate constant $\langle k\rangle$ and to the variance σ^2 of the rate distribution function by the relations

$$\langle k\rangle = nk_o \qquad \sigma^2 = nk_o^2 \tag{5}$$

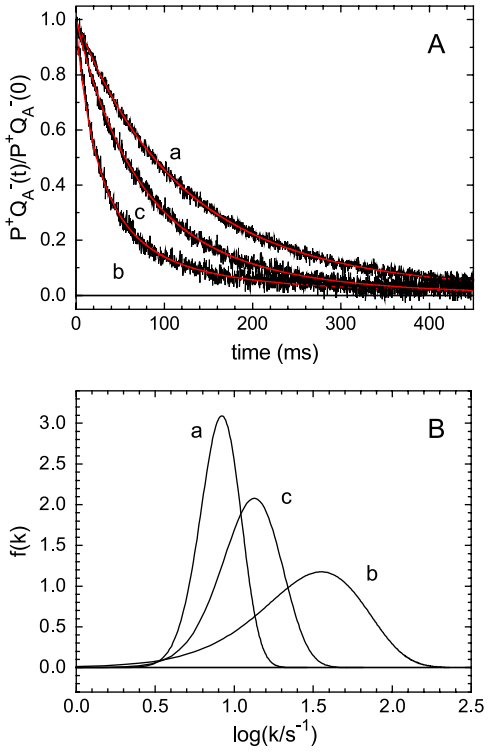

Fig. 1. Kinetic analysis of $P^+Q_A^-$ charge recombination following laser flash excitation of RC in a trehalose solution and in dehydrated amorphous trehalose matrices. (A) Normalized decay of the $P^+Q_A^-$ state measured in a 0.4 M trehalose solution (trace a), in a dry solid trehalose matrix, characterized by 4700 H_2O molecules per RC (trace b) and following partial rehydration of the glass at 7800 H_2O molecules per RC by exposure to water vapor (trace c). Traces are the average of four measurements at 450 nm. Although for the sake of visual clarity, only the first 450 ms of $P^+Q_A^-$ decays are shown, the time scale of kinetic recording extended out to 1 s after the laser pulse and the whole information was used when fitting kinetics to Eq. (4) (see red continuous curves). This procedure and Eq. (5) yield the following average rate constant and distribution width: $\langle k\rangle = 8.3$ s^{-1}, $\sigma = 2.5$ s^{-1} for trace a; $\langle k\rangle = 35.5$ s^{-1}, $\sigma = 26.5$ s^{-1} for trace b; $\langle k\rangle = 13.4$ s^{-1}, $\sigma = 5.8$ s^{-1} for trace c. (B) The corresponding distribution functions $f(k)$, defined on a logarithmic scale [$p(k)dk = f(k)$ d log(k)], calculated according to Eq. (3), using the parameter values obtained from fitting traces to a power law (see above).

Fig 1 shows the decay kinetics of laser induced $P^+Q_A^-$ (panel A) and the corresponding rate distribution functions (panel B) obtained in a trehalose solution (a), in a dehydrated solid trehalose–water matrix (b), and upon partial rehydration of the dried glass (c). Incorporation of the RC into a strongly dehydrated trehalose–water matrix leads to a substantial acceleration of the decay kinetics and to a dramatic broadening of the rate distribution function. Both effects revert upon rehydration (c). As discussed in detail in Ref. [18], both the acceleration and the spreading of the kinetics

of $P^+Q_A^-$ recombination observed at room temperature upon drying of trehalose–water matrices are in remarkable agreement with those detected upon cooling the RC to cryogenic temperatures [11,14]. Based on this similarity, we interpret the accelerated and distributed kinetics observed in dry trehalose matrices within the framework of the dynamic model put forward by McMahon et al. [14]. In solid trehalose–water matrices, the RC relaxation from the dark-adapted to the light-adapted conformation, which stabilizes primary charge separation in solution at room temperature, is drastically slowed down over the time scale of $P^+Q_A^-$ recombination. This results in an accelerated $P^+Q_A^-$ recombination, occurring in a structurally inhomogeneous population of essentially nonrelaxed dark-adapted proteins. A quasi-static structural heterogeneity of the RC is revealed by the large broadening of the distribution of electron transfer rates, which results from the trapping of conformational substates in the more dry matrices (Fig. 1B, distribution b). At variance, in relatively wet glasses, interconversion between substates and protein relaxation, although slowed down as compared to solution, take place over the time scale of charge recombination. This leads to partial averaging of the static conformational heterogeneity of RCs [narrowing of $p(k)$] and partial stabilization of the charge separated state (decrease of $\langle k \rangle$) (see Fig. 1B, distribution c).

To further substantiate this interpretation and to get information on the residual conformational flexibility of the RC incorporated into dry trehalose matrices, we studied the kinetics of $P^+Q_A^-$ recombination following photoexcitation of the sample by continuous illumination (lasting from a few milliseconds to tens of seconds). The underlying idea is that, even with a protein dynamics strongly inhibited on the tens of milliseconds time scale, when the RC experience the charge separated state for a sufficiently long spell of time, the protein–water–trehalose structures present in the sample [26,30] can undergo structural rearrangements stabilizing $P^+Q_A^-$. The occurrence of these slow conformational rearrangements, taking place over the time of continuous illumination, can be probed by the kinetics of $P^+Q_A^-$ recombination, which appear to be very sensitive to the conformational state and dynamics of the RC.

Fig. 2A compares kinetic traces of $P^+Q_A^-$ recombination measured following a laser pulse (trace a) and a 2-s period of continuous illumination (trace b) in a dry trehalose matrix, characterized by a water to RC molar ratio of 5.9×10^3. Fig. 2B gives the corresponding rate distributions, calculated on the basis of k_o and n values obtained by fitting the traces to Eq. (4) (see continuous lines in Fig. 2A). The value of the average rate constant, $\langle k \rangle = 29.0\ s^{-1}$ following the 20-ns laser pulse, decreases to $16.7\ s^{-1}$ when charge recombination is examined after a 2-s continuous photoexcitation. This slowing of the reaction is accompanied by a significant narrowing of the distribution of electron transfer rates: the width σ of $p(k)$ decreases from $20.0\ s^{-1}$ following laser excitation to $7.7\ s^{-1}$ following continuous photoexcitation of the RC.

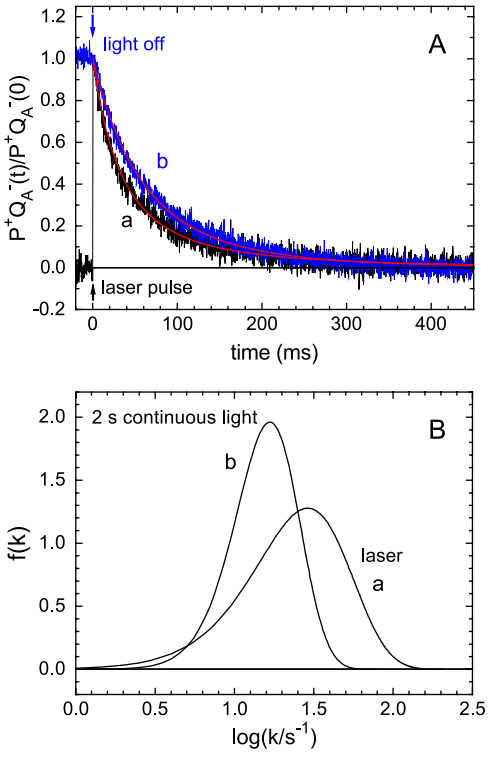

Fig. 2. The effect of continuous illumination on $P^+Q_A^-$ recombination kinetics in a dried trehalose matrix characterized by 5900 H_2O molecules per RC. Normalized kinetic traces measured following a laser pulse (a) and 2-s continuous illumination (b) are shown in panel A. The abscissa represents the time after the laser flash and after closure of the shutter gating continuous light, respectively. Traces are the average of four measurements at 450 nm. Decay kinetics were recorded over a time scale of 1 s and the whole kinetic information was processed (see text for details). Best fitting to Eq. (4) (red continuous curves) and Eq. (5) yield $\langle k \rangle = 29.0\ s^{-1}$, $\sigma = 20.0\ s^{-1}$ (trace a), and $\langle k \rangle = 16.7\ s^{-1}$, $\sigma = 7.7\ s^{-1}$ (trace b). The corresponding distribution functions $f(k)$ (see the legend of Fig. 1) are presented in panel b.

Although for the sake of visual clarity, $P^+Q_A^-$ decays are shown in Figs. 1A and 2A only over a 0.45-s time interval, data acquisition always extended out to at least 1 s, i.e., to a time at which, in trehalose–water matrices under all conditions studied, the fraction of survived $P^+Q_A^-$ was less than 10^{-3}. To improve confidence in the determination of the rate distribution functions (particularly of their widths) the whole kinetic information was always used when fitting decays to Eq. (4). In continuous-light measurements, the time course of absorbance change was routinely recorded from the onset of photoexcitation and therefore included the formation of the charge separated state $P^+Q_A^-$, which is maintained at a steady level during continuous illumination. These data (not shown in Fig. 2A, where only part of the recorded kinetics is presented) clearly indicate that, even following the longest light exposure, a complete decay of the $P^+Q_A^-$ signal occurs over 1 s after gating off the light. The absence of a detectable offset in the recovery kinetics suggests that no trapping of the electron on Q_B nor any other side reaction interfering with $P^+Q_A^-$ recombination and

involving separately the primary donor and/or acceptor takes place.

The results of a series of measurements performed on the same trehalose matrix at different durations of the continuous photoexcitation are summarized in Fig. 3A,B (filled circles). The figure also shows data from independent experiments performed on two distinct samples, characterized by a similar H_2O/RC ratio (filled squares and diamonds). In all sets of data, increasing the photoexcitation time from a few milliseconds to tens of seconds makes the kinetics of $P^+Q_A^-$ recombination to become progressively slower and less distributed. Most of the effect, both on $\langle k \rangle$ (panel A) and on σ (panel B), occurs within a few seconds of continuous illumination.

Because the recombination kinetics is extremely sensitive to the content of residual water of the trehalose matrix (see Fig. 1 and Ref. [18]), the hydration state of the glass during the whole course of the experiment was systematically checked by alternating measurements after continuous illumination with the acquisition of kinetic traces induced by a laser pulse in the dark-adapted sample. These last kinetics yielded systematically the same rate distribution within the experimental error, thus excluding variations in the water content of the sample during the time of the experiment or memory effects due to the long light exposures. In this respect, we note that recovery of the dark-adapted state following exposures to continuous light for tens of seconds was completed in less than 10 s, i.e., on a time scale comparable to that of the transition from the dark- to the light-adapted conformation during continuous illumination. This was tested by monitoring the kinetics of recombination of the $P^+Q_A^-$ state induced by a single laser pulse fired 10 s after switching off the continuous illumination. The same recombination kinetics measured in the sample dark-adapted for minutes were always observed (not shown).

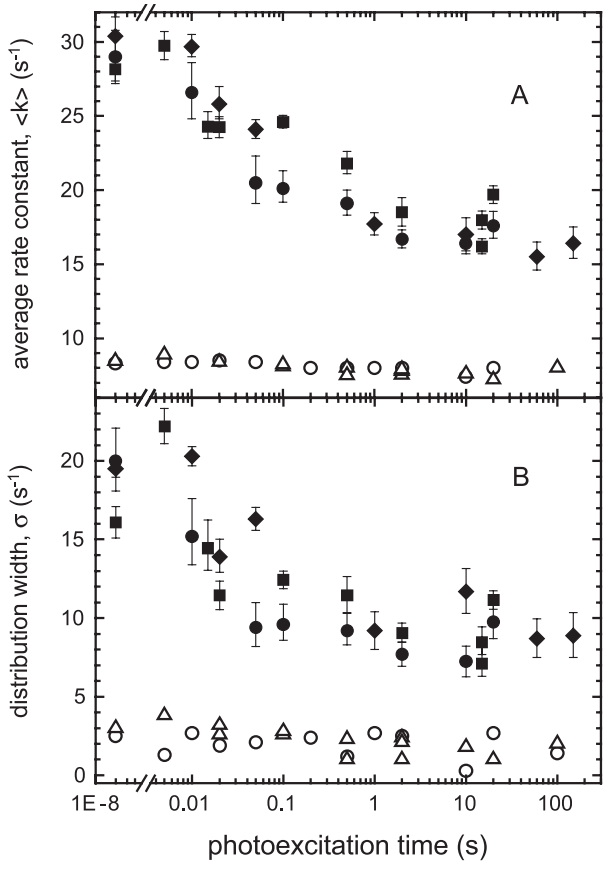

Fig. 3. Dependence of the kinetics of $P^+Q_A^-$ recombination upon the duration of continuous illumination measured in dry trehalose matrices and in trehalose solutions. Kinetic analysis was performed as illustrated in Fig. 2 and detailed in the text. Values of the average rate constant $\langle k \rangle$ and of the distribution width σ are shown for three dehydrated glasses, characterized by a similar content of residual water: H_2O/RC = 5900 (filled circles), H_2O/RC = 5400 (filled diamonds), and H_2O/RC = 5600 (filled squares). As a control, kinetics were analyzed in a liquid 0.4 M trehalose solution (open circles) and following resolubilization of a dried (H_2O/RC = 5900) trehalose matrix at the end of a continuous-light experiment (open triangles). Vertical bars give the confidence intervals within two standard deviations.

In control measurements performed in liquid detergent suspensions of RCs, the kinetics of $P^+Q_A^-$ recombination was essentially not affected by the duration of photoexcitation. In such experiments, values of $\langle k \rangle$ and σ fluctuate around 8 and 2 s^{-1}, respectively (see Fig. 3A and B, open circles). It appears therefore that the marked progressive decrease observed in $\langle k \rangle$ and σ following photoexcitation of increasing duration is a peculiar feature of RCs embedded into a dry trehalose matrix and not an intrinsic property of the RC in solution. Moreover, when the amorphous matrix is redissolved after a continuous light experiment, the kinetics of $P^+Q_A^-$ recombination originally observed in solution and independent of the duration of continuous preillumination is fully restored (see Fig. 3A,B, open triangles).

From the data of Fig. 3, we infer that in the dry solid trehalose matrix, the persistence of the primary charge separated state induced by a continuous intense illumination leads to partial slow relaxation of the RC protein–solvent system that stabilizes the $P^+Q_A^-$ state (decrease of $\langle k \rangle$). This process appears to be coupled to narrowing of the rate distribution function.

4. Discussion

Several studies, mostly performed on carbon monoxy myoglobin, have been addressed to characterize the inhibition of internal protein dynamics caused by incorporation of a soluble protein into room temperature amorphous trehalose–water matrices. In particular, in extremely dry matrices, large hindering has been reported for the nonharmonic contributions to the mean square displacement of the hydrogen atoms even at room temperatures [22]. Accordingly, large-scale motions of the iron atom, detected by Mössbauer spectroscopy in hydrated myoglobin samples above $\sim$ 180 K, are severely damped in trehalose–water matrices of low water content [21]. In full agreement, under conditions of

drought, thermal interconversion among conformational A substates is almost fully arrested and the kinetics of CO rebinding are governed by a quasi-static distributions of rate constants [24]. These observations point to a tight structural and dynamic coupling between the protein and the external trehalose–water matrix, as recently evidenced [30].

Insertion into a dehydrated trehalose matrix strongly reduces room temperature dynamics also in a large membrane protein as the photosynthetic RC [18,19]. The kinetics of primary charge recombination has been analyzed in trehalose solutions and amorphous matrices upon systematically increasing the trehalose/water ratio [18]. Above 90 wt.% trehalose, where the sample turns into a solid matrix, we found a drastic increase in the average rate constant ($\langle k \rangle$) and a pronounced broadening of the rate distribution function. This behavior is expected for nonhomogeneous protein samples in which both thermal fluctuations between conformational substates and RC relaxation to the light-adapted charge separated state are drastically slowed over the time scale of $P^+Q_A^-$ recombination.

In the driest solid samples (98 wt.% trehalose), values of $\langle k \rangle$ and of the distribution width (σ) close to 27 and 16 s^{-1}, respectively, were attained in Ref. [18]. The data of Fig. 1 confirm and extend these observations. In the present work, a more efficient room temperature trapping of RC conformational substates has been obtained by decreasing the trehalose to RC molar ratio in the matrix from 1.5×10^4 [15] to 1.0×10^4 (see Materials and methods). Under these conditions, matrices can be routinely dehydrated to a stable state, characterized by a water to RC ratio $\approx 5 \times 10^3$, in which $P^+Q_A^-$ recombination kinetics are described by $\langle k \rangle \approx 35$ and $\sigma \approx 20$ s^{-1}. Even larger effects ($\langle k \rangle \approx 50$, $\sigma \approx 40$ s^{-1}) were detected occasionally (not shown), presumably as a result of further depletion of residual bound water. However, such extremely dried states could not be easily maintained during optical measurements even under continuous N_2 flow and their water content could not be safely evaluated by NIR spectroscopy due to slight water uptake during measurements.

Recent results of molecular dynamics simulations [26] indicate that in trehalose amorphous matrices, myoglobin is constrained within a network of hydrogen bonds connecting protein surface groups, water, and sugar molecules, and that the fraction of water molecules involved in hydrogen bonds with both protein and sugar increases with dehydration. Most likely, therefore, the trehalose–water matrix restricts protein conformational dynamics by locking the protein surface topology. Indeed, on the basis of a vibrational echo study, Rector et al. [31] have recently suggested that structural fluctuations involving displacements of the protein surface are strongly coupled to the viscoelastic response of the trehalose matrix. In view of the above considerations, it is not surprising that limited changes in the composition of the trehalose–water–protein structures (e.g., in trehalose/RC ratio) can sizably affect the efficacy of the solid dehydrated matrix in conditioning RC dynamics.

To compare quantitatively our rate distributions with those determined at low temperatures [14], we switch from $p(k)$ of Eq. (3) to the distribution function $f(k)$ defined on a logarithmic scale [being $p(k)\ \mathrm{d}k = f(k)\ \mathrm{d}\ \log(k)$]. With reference to the data of Fig. 1, in the solid dehydrated trehalose matrix, characterized by a residual water content corresponding to 4700 water molecules per RC, $\langle \log (k/s^{-1}) \rangle = \int \log(k)\ f(k)\ \mathrm{d}\ \log(k)$ is 1.4. This value compares with $\langle \log (k/s^{-1}) \rangle = 1.6$ in RCs cooled in the dark at $T = 5$ K, indicating that relaxation from the dark-adapted to the light-adapted conformation is drastically hindered at room temperature following a ns laser photoexcitation on the time scale of $P^+Q_A^-$ recombination. The width of the rate distribution, defined as $[\int f(k)\ (\log(k) - \langle \log(k) \rangle)^2\ \mathrm{d}\ \log(k)]^{1/2}$, is 0.4 in the room temperature trehalose glass of Fig. 1 (distribution b). The broadening observed in room temperature trehalose matrices is considerably larger than that detected upon cooling the RC to 5 K in a glycerol–water mixture in the dark (0.2 decades) [14]. This shows that fluctuations between conformational substates no longer occur in the trehalose matrix and that the RC static heterogeneity observable in trehalose is different from and more pronounced than that revealed by freezing RC to cryogenic temperature in the dark [11,14]. Indeed, in the two cases, trapping of RC conformations occurs following a quite different evolution of the system resulting in different physical conditions. In the latter case, the temperature of the water–glycerol mixture is decreased at a rate of ≈ 20 K/min [14], while in the former, locking of RC conformational dynamics is caused by a dramatic perturbation of the protein energy landscape, which, however, is established very slowly (during days) at 30 °C as a consequence of progressive dehydration of the sample (see Materials and methods).

Kriegl et al. [32] have recently compared the temperature dependence of $P^+Q_A^-$ recombination kinetics in cryosolvent and in a sol–gel matrix. Following encapsulation of the RC into a silica matrix, the rate distribution at room temperature is strongly broadened, to an extent comparable to that observed in dried trehalose matrices. Interestingly, however, in this matrix, the rate of charge recombination was not increased at room temperature, but rather decreased by a factor of ≈ 2. It appears therefore that, in contrast to that observed in trehalose-coated RCs, the room temperature relaxation to the light-adapted state is not slowed down for RCs embedded in the hydrogel. A further interesting behavior is observed when the RC is dried in the absence of trehalose or when it is incorporated into polyvinyl alcohol matrices: in both cases, $P^+Q_A^-$ recombination kinetics are scarcely affected, even at extremely low amounts of residual water [18, 33]. The above-mentioned results suggest that the structural details of the protein–matrix interaction largely and specifically contribute to determine RC internal motions.

Preillumination of trehalose-coated RCs by exposure to continuous light allows to extend the time range over which the occurrence of conformational changes can be probed.

By exploiting this strategy, we characterized the inhomogeneous inhibition of Q_A^--to-Q_B electron transfer in moderately dehydrated trehalose matrices [19]. In the present paper, kinetic analysis of $P^+Q_A^-$ recombination has been used to probe protein relaxation that occurs during prolonged expositions to continuous light when RCs are embedded into dry trehalose matrices. In dry glasses, when the duration of photoexcitation is increased from 2×10^{-8} s (laser pulse) to the second time scale, recombination kinetics of $P^+Q_A^-$ slows down progressively and becomes less distributed (Fig. 3). It appears that, even in dry solid matrices, some internal protein motions are allowed, which, when the electric field perturbation induced by $P^+Q_A^-$ persists, lead to progressive stabilization of the charge separated state. The minimum value of $\langle k \rangle$ attained following continuous illumination is intermediate between that measured in solution and in the dry glass following a laser pulse, indicating that, during the time of continuous photoexcitation, the RC protein undergoes conformational relaxation, which, however, does not fully stabilizes $P^+Q_A^-$. Increasing the time of photoexcitation beyond a few seconds has no or very limited further effects on the rate distribution functions, suggesting that in dry matrices, the light-adapted conformations promptly attained in solution are precluded also on the time scale of tens of seconds.

Interestingly, the decrease in $\langle k \rangle$ is paralleled by a decrease of the width σ of the rate distribution, which again saturates at values intermediate between those observed in solution and those corresponding to a quasi-static distribution. When RCs are cooled to cryogenic temperature in the light, the broadening of the rate distribution is markedly larger than upon cooling in the dark, suggesting a more pronounced static heterogeneity of the RC population in the light-adapted conformation [11,14,32]. In view of this, it seems unlikely that the narrower rate distribution observed following continuous illumination reflects a reduced quasi-static heterogeneity of the light-adapted state reached in the dry matrix. Rather, we believe that narrowing of the rate distribution indicates a partial averaging of conformational substates over the time scale of charge recombination, which becomes faster as the duration of photoexcitation is increased. We propose that, when the electric field perturbation generated by primary charge separation is maintained for a sufficiently long time, the continuous attempts of the protein to undergo conformational changes toward the more stable light-adapted state, make to collapse the hydrogen bond network connecting the protein surface to the matrix [26,30]. This, in turn, results in partial unlocking of the protein surface and increases the protein motional freedom. During continuous illumination, the RC protein is gaining a limited conformational flexibility that allows both partial relaxation (decrease of $\langle k \rangle$) and substate averaging (decrease of σ). A similar parallelism between $\langle k \rangle$ and σ variations also appears when analyzing $P^+Q_A^-$ recombination in glasses characterized by a variable content of residual water (see Ref. [18] and Fig. 1B), suggesting that, in contrast to what found in hydrogels, protein substates fluctuations and motions solvating the charge separated state are closely connected in solid trehalose glasses. As a whole, therefore, the present data support the notion of a tight dynamical coupling within protein–water–trehalose structures [30].

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