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Electron-Transfer Kinetics in Photosynthetic Reaction Centers Cooled to Cryogenic Temperatures in the Charge-Separated State: Evidence for Light-Induced Structural Changes†

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ABSTRACT: We have compared the electron-transfer kinetics in reaction centers (RCs) cooled in the dark with those cooled under illumination (i.e., in the charge-separated state). Large differences between the two cases were observed. We interpreted these findings in terms of light-induced structural changes. The kinetics of charge recombination $D^+Q_A^- \rightarrow DQ_A$ in RCs containing *one quinone* were modeled in terms of a distribution of donor–acceptor electron-transfer distances. For RCs cooled under illumination the distribution broadened and shifted to larger distances compared to the distribution for RCs cooled in the dark. The model accounts for the nonexponential decay observed at low temperatures [McElroy, J. D., Mauzerall, D. C., & Feher, G. (1974) *Biochim. Biophys. Acta 333*, 261–277; Morrison, L. E., & Loach, P. A. (1978) *Photochem. Photobiol. 27*, 751–757]. A possible physiological role of the structural changes is an enhanced charge stabilization. For RCs with *two quinones*, the recombination kinetics $D^+Q_AQ_B^- \rightarrow DQ_AQ_B$ were found to be strongly temperature dependent. This was interpreted in terms of temperature-dependent transitions between structural states [Agmon, N., & Hopfield, J. J. (1983) *J. Chem. Phys. 78*, 6947–6959]. This interpretation requires that these transitions occur at cryogenic temperatures on a time scale $t \gtrsim 10^3$ s. The electron transfer from Q_A^- to Q_B was found to not take place in RCs cooled in the dark ($\tau_{AB}^{dark} > 10^{-1}$ s). In RCs cooled under illumination, we found $\tau_{AB}^{light} < 10^{-3}$ s. We suggest the possibility that the drastic decrease in τ_{AB} observed in RCs cooled under illumination is due to the trapping of a proton near Q_B^-.

The primary process in photosynthesis involves the conversion of light into electrochemical energy through the formation of oxidized and reduced molecules. In photosynthetic bacteria, this process occurs in the reaction center (RC),[1] a protein complex that spans the plasma membrane. The RC consists of three polypeptide subunits and a number of cofactors associated with the electron-transfer chain: four bacteriochlorophylls, two bacteriopheophytins, two ubiquinones (UQ-10), and one non-heme iron (Fe^{2+}) [for a review, see Feher & Okamura (1978)]. The light induces a charge separation with an electron leaving the donor, D, a specialized bacteriochlorophyll dimer, and passing via intermediates to the primary and secondary quinone acceptors, Q_A and Q_B, respectively [for a review, see Parson & Ke (1982)].

In this work we address two questions concerning the structural dynamics of RCs: (1) Is the light-induced charge separation accompanied by a change in the structure of the RC? (2) Do the RCs have a unique structure, or are they distributed over a range of structural states?

The possibility of a light-induced structural change was discussed by McElroy et al. (1974). They found that the oxidized donor (i.e., D^+) was trapped in RCs cooled to cryogenic temperatures after a long period of illumination and suggested that this could result from immobilizing RCs in a conformation favoring the charge-separated state. Similar findings were reported by Noks et al. (1977, and references therein) and by Ke et al. (1979) for photosystem I of chloroplasts [for a review of early work, see Ke et al. (1976)]. Evidence for bulk structural changes comes from the calorimetric studies of Arata & Parson (1981a,b). Their data suggested that the light-induced charge separation is accompanied by a decrease in the volume of the RC–solvent system. However, Kirmaier et al. (1983) found from photodichroism measurements that the bacteriochlorophylls and bacteriopheophytins did not move significantly with respect to each other in the time interval between 2 ns and 10 ms after excitation.

A search for specific light-induced structural changes using protein modification techniques was performed by Noks et al. (1977). They found that incubation of chromatophores with glutaraldehyde, a cross-linker of amino groups, affected the electron-transfer kinetics in RCs only when the incubations were performed under illumination. Since the kinetics are

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‡ Work performed in partial fulfillment for the Ph.D. degree.

[1] Abbreviations: LDAO, lauryldimethylamine *N*-oxide; LN_2, liquid nitrogen; RC, reaction center; Tris, tris(hydroxymethyl)aminomethane; UQ, ubiquinone.

0006-2960/84/0423-5780$01.50/0

expected to be sensitive to details of the RC structure, this result suggests that there is a light-induced change in the protein structure, which makes an amino group accessible to cross-linking.

The possibility that RCs exist in a distribution of structural states is part of a broader problem in protein dynamics [for review, see Karplus & McCammon (1981), Debrunner & Frauenfelder (1982), and Hopfield (1984)]. Comprehensive evidence that proteins exist in such distributions comes from the work of Austin et al. (1975) and Frauenfelder (1978), who studied the dynamics of ligand binding to heme compounds. Recent studies by Woodbury & Parson (1984) suggest that there is a discrete distribution of states involved on the time scale (t <10 ns) of the early electron-transfer steps in RCs.

We approached the study of structural dynamics by measuring the kinetics of charge separation and recombination. These kinetics depend strongly on the three-dimensional configuration of the reactants and, therefore, serve as a sensitive probe for studying structural changes. Light-induced structural changes may be trapped when RC conformations are immobilized at low temperature. Thus, a comparison of the kinetics of RCs cooled to cryogenic temperature under illumination, i.e., in the charge-separated state, with those cooled in the dark should give an answer to the questions raised above.

The kinetics were measured optically in two systems. The first involved RCs with one quinone (1UQ/RC; i.e., Q_B removed) in which the charge-separated state $D^+Q_A^-$ can be formed at all temperatures (Parson, 1967; McElroy et al., 1974; Loach et al., 1975). The second invovled RCs with both quinones (2UQ/RC) in which electron transfer from Q_A^- to Q_B is observed at room temperature, but normally is not observed in RCs cooled to cryogenic temperatures in the dark (Parson, 1978).

The results of the kinetic measurements were analyzed in terms of a simple structural model as a means of estimating both the extent of the light-induced structural changes and the distribution of structures involved in the two systems. The results of this analysis were compared with those found by structural studies on other proteins (Austin et al., 1975; Frauenfelder et al., 1979; Artymiuk et al., 1979; Karplus & McCammon, 1981).

A preliminary account of this work has been presented earlier (Kleinfeld et al., 1983).

Materials and Methods

Reaction Centers. RCs were isolated from *Rhodopseudomonas sphaeroides* R-26 as previously described (Feher & Okamura, 1978). RCs with one or fewer quinones were prepared by the method of Okamura et al. (1975). The number of quinones was determined by a cytochrome c photooxidation assay as previously described (Okamura et al., 1982). The 2UQ/RCs averaged 1.95 ± 0.05 quinones and the 1UQ/RCs averaged 0.76 ± 0.04 quinones. The low quinone content for the 1UQ/RC sample minimized the equilibrium fraction of RCs having two quinones; this fraction was determined to be 0.03 ± 0.01 by a multiple flash cytochrome c assay (Parson, 1969; Kleinfeld et al., 1984). RC concentrations were determined by using the extinction coefficient $\epsilon^{800} = 2.88 \times 10^5$ M^{-1} cm^{-1} (Straley et al., 1973).

All measurements unless otherwise specified were performed with RCs in 10 mM Tris-HCl, 0.025% (w/v) LDAO, and 50% (v/v) glycerol, pH 8.0.

Cryogenic Techniques. Samples were placed in a 1-mm path length cell (made with 0.15 mm thick glass cover slides held by vacuum grease to a copper frame) and dark adapted for at least 5 min before cooling. Samples cooled in the dark were plunged into liquid nitrogen (LN_2). Those cooled under illumination were irradiated typically for 1 s at room temperature. A LN_2-filled Dewar with optically transparent windows was then raised over the sample while the illumination was continued. Samples reached 77 K in 3–5 s, forming a clear glass with a few fine cracks. For measurements at 77 K the samples were transferred to an optical Dewar filled with LN_2, with the sample in contact with the liquid through a cold finger. For measurements at other temperatures the samples were transferred to a variable temperature cryostat (Oxford Instruments CF-204), in which the temperature was maintained through a helium exchange gas. The sample temperature was continuously monitored with either a platinum or carbon resistance thermometer in contact with the sample holder. Temperature variations were less than ±0.2 K during an experiment.

Optical Techniques. Rapid changes in the absorption spectrum were recorded with a spectrophotometer of local design (Kleinfeld et al., 1984) that had a time resolution of 0.5 μs. Slower changes (t >10 s) were recorded with a Cary 14R spectrophotometer. Care was taken to limit the intensity of the monitoring beam to prevent bleaching of the RCs during a measurement. Flash-induced charge separation was accomplished by cross illuminating the sample (mounted at 45° relative to the measuring beam) with a dye laser (Phase-R DL2100C, λ = 584 nm, 0.4 μs pulse, 0.2 J per pulse). Continuous illumination was provided by a tungsten lamp filtered by 2 cm of water and a Corning CS2-64 filter (I = 1.1 W cm^{-2}).

Experimental Results

(A) RCs with One Quinone

The charge separation for RCs with one quinone is described by

$$DQ_A \underset{\tau_{AD}}{\overset{h\nu\ \tau_{h\nu}}{\rightleftharpoons}} D^+Q_A^- \qquad (1)$$

The formation and subsequent decay of $D^+Q_A^-$ is measured by monitoring the RC absorption peak at 865 nm at room temperatures and at 890 nm at cryogenic temperature (Feher, 1971). This peak is bleached after a laser flash due to the formation of D^+, and recovers with the characteristic time τ_{AD}.[2] Light-induced generation and recombination of the charge-separated state $D^+Q_A^-$ have been observed from room temperature down to 1.6 K (McElroy et al., 1974). At room temperature, $\tau_{AD} \sim 10^{-1}$ s (Parson & Ke, 1982).

Charge Recombination Kinetics at 77 K. The flash-induced absorption changes, $\Delta A(t)$, for RCs cooled to 77 K are shown in Figure 1. When the RCs were cooled in the dark, the charge recombination time[2] was

$$\tau_{AD}^{dark} = 25 \text{ ms}$$

in agreement with previous reports (McElroy et al., 1974; Parson, 1967; Loach et al., 1975; Morrison & Loach, 1978). When the same RCs were warmed to room temperature and recooled under continuous illumination (charge separation time $\tau_{h\nu}$ = 0.8 ms, see eq 1), the recombination time[2] changed to

$$\tau_{AD}^{light} = 120 \text{ ms}$$

When the sample temperature was maintained at 77 K, τ_{AD}

[2] The recombination time, τ_{AD}, is defined as the time it takes for the absorption change to decay to $1/e$ of its maximum value. For a simple first order process τ_{AD} equals the inverse of the rate constant, i.e., k_{AD}^{-1}.

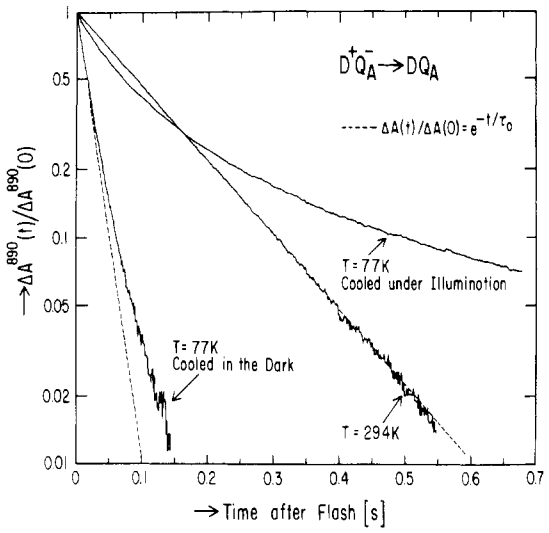


FIGURE 1: Semilog plot of the electron donor recovery kinetics in 1UQ/RC samples following a laser flash. $\Delta A^{890}(0)$ for RCs cooled under illumination was essentially the same as that for RCs cooled in the dark. The room temperature kinetics (T = 294 K, monitored at 865 nm) were corrected by subtracting a slowly decaying component [3.3% of $\Delta A^{865}(0)$ with a time constant of 1.52 s], caused by the presence of RCs with two quinones (see Materials and Methods). Dashed lines represent fits to an exponential function, with τ_0 = 22 ms for RCs cooled to 77 K in the dark and τ_0 = 132 ms for RCs at 294 K. Note the large deviation from an exponential of the kinetics in RCs cooled under illumination. RC concentrations were 19 μM for 77 K experiments and 3.8 μM for 294 K experiment.

remained unchanged for at least 50 h, which was the longest time measured. When the sample was warmed to room temperature and recooled in the dark, the original kinetic behavior (τ_{AD}^{dark} = 25 ms) was restored. This shows that the illumination caused no permanent change in the RCs.

The recombination kinetics for RCs cooled in the dark showed small deviations from an exponential time dependence (Figure 1) as previously reported (McElroy et al., 1974; Morrison & Loach, 1978). This deviation was found to be much more pronounced in RCs cooled under illumination (Figure 1).

To determine if the nonexponential behavior observed at cryogenic temperatures persisted for RCs at room temperatures, the charge recombination kinetics were measured at 294 K under identical buffer conditions. A pure exponential recovery (τ_{AD} = 132 ms) was observed over 2 orders of magnitude in amplitude (Figure 1).

To determine the effect of protonation on the recombination kinetics, both τ_{AD}^{dark} and τ_{AD}^{light} were measured as a function of pH ($6.0 \leq pH \leq 11.2$) at T = 77 K. For RCs cooled in the dark there was no effect while for RCs cooled under illumination there was a small pH dependence that could be fitted with the relation $\tau_{AD}^{light} \propto [H^+]^{0.045}$.

The characteristic recombination time for RCs cooled under illumination varied within $\pm$10% between samples. This variation may have been caused by small differences in the cooling rate between samples. No systematic changes in the kinetics were found by varying the room temperature illumination period between 1 and 10 s. No variations in the kinetics were found with RCs cooled in the dark.

Temperature Dependence of the Recombination Kinetics. The recombination kinetics of $D^+Q_A^-$ for RCs cooled in the dark remained essentially unchanged when the temperature was lowered from ~80 to 4 K (see Figure 2). These results are in agreement with previous work on RCs and chromatophores from *R. sphaeroides* (McElroy et al., 1974). For chromatophores from other bacteria a small temperature

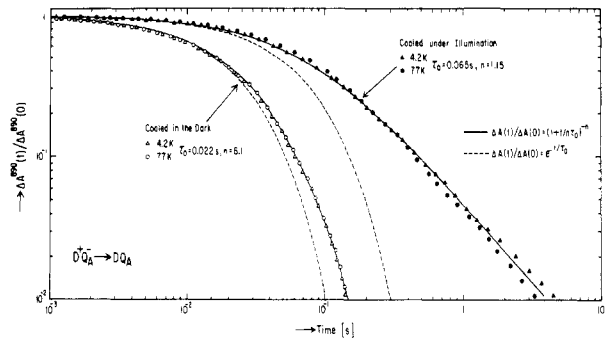


FIGURE 2: log–log plot of the donor recovery kinetics at 4.2 and 77 K in 1UQ/RC samples cooled in the dark and under illumination. Dashed lines represent fits of the initial slopes of the data to an exponential; solid lines are fits to a power law (eq 2). The values of parameters τ_0 and n are given in the figure. Note that τ_0 is the same for both functions. RC concentration was 19 μM.

dependence has been reported (Parson, 1967; Loach et al., 1975). When RCs were cooled under illumination, τ_{AD}^{light} changed slightly as the temperature was lowered from 80 to 4 K (Figure 2).

The kinetic data deviated significantly from an exponential (see dashed line, Figure 2) but could be well fitted with the function

$$\Delta A(t)/\Delta A(0) = [1 + t/(n\tau_0)]^{-n} \qquad (2)$$

where τ_0 and n are adjustable parameters (see solid line, Figure 2). This function, used by Austin et al. (1975) for their ligand binding results, has the advantage of being amenable to an exact mathematical analysis; i.e., it is Laplace transformable in closed form (see Theoretical Model).

When the sample temperature was raised above ~90 K, τ_{AD}^{dark} increased monotonically with increasing temperature, in agreement with previous reports (Parson, 1967; Loach et al., 1975; Hsi & Bolton, 1974; Mar et al., 1983). This increase was stable with time at each temperature and was completely reversible as the temperature was cycled.

For RCs cooled under illumination, a different behavior of the kinetics was observed: Below ~90 K, τ_{AD}^{light} remained constant with time at given temperature. Above ~90 K, τ_{AD}^{light} decreased with time, heading toward the value of τ_{AD}^{dark}. Apparently, the structural changes that had been frozen in during illumination were annealing out at $T > 90$ K.

(*B*) *RCs with Two Quinones*

The kinetic properties of this system are described by

$$DQ_AQ_B \underset{\tau_{AD}}{\overset{h\nu}{\rightleftharpoons}} D^+Q_A^-Q_B \underset{\tau_{BA}}{\overset{\tau_{AB}}{\rightleftharpoons}} D^+Q_AQ_B^- \qquad (3)$$
$$\tau_{BD}$$

Electron transfer from Q_A^- to Q_B occurs in $\tau_{AB} \sim 10^{-4}$ s at room temperature (Vermeglio & Clayton, 1977); the decay of the state $D^+Q_AQ_B^-$ occurs with a time $\tau_{BD} \sim 1$ s (Okamura et al., 1982). As in the case of RCs with one quinone, the recombination kinetics were measured by monitoring the optical absorption peak of the donor at 890 nm (T = 77 K).

Charge Recombination Kinetics at 77 K. When RCs containing two quinones were cooled in the dark, the charge recombination following a laser flash exhibited the same kinetics as found with RCs containing only a single quinone. The reason is that the electron transfer from Q_A^- to Q_B is not observed at cryogenic temperatures; one measures, therefore, the decay of the $D^+Q_A^-Q_B$ state.

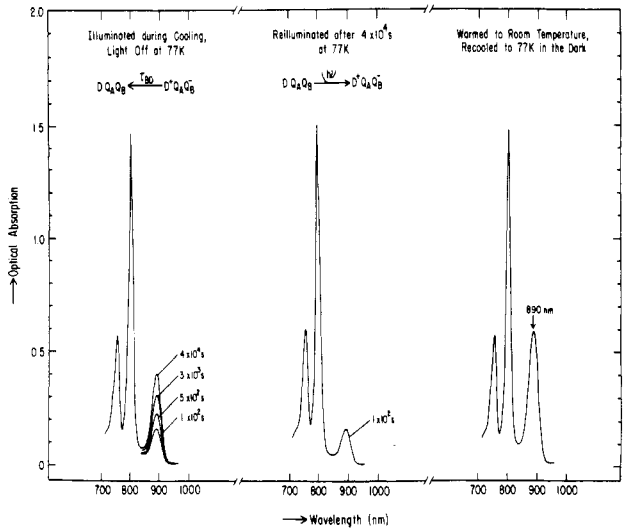
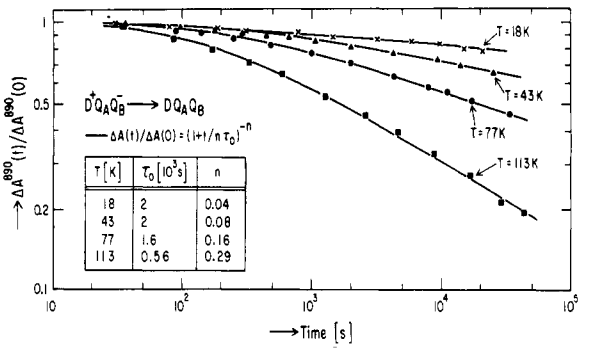


FIGURE 3: Near-infrared absorption spectra of 2UQ/RC samples at 77 K. Indicated times refer to time intervals after cessation of illumination. Reillumination of sample was performed with 10 s of continuous illumination. The kinetics at different temperatures are presented in Figure 4. RC concentration was 23 μM.



T[K]	τ_0[10^3s]	n
18	2	0.04
43	2	0.08
77	1.6	0.16
113	0.56	0.29

FIGURE 4: log–log plot of the donor recovery kinetics at different temperatures in 2UQ/RC samples cooled under illumination. Data were normalized to the maximum absorption change, $\Delta A^{890}(0)$, found by extrapolating the measured absorption changes back to zero time (more data were acquired at short times than are shown). The maximum absorption level [i.e., $A^{890}(\infty)$], which served as the base line for the absorption changes, was determined by warming and recooling the sample in the dark. Typically, $\Delta A^{890}(0)$ was 80% of $A^{890}(\infty)$. The parameters τ_0 and n were found from fitting the data to eq 2 and are tabulated in the insert. RC concentration was 23 μM.

In order to study the recombination kinetics of $D^+Q_AQ_B^-$ at low temperature, this state was trapped at 77 K by cooling RCs under illumination (see Figure 3). The two main findings of these experiments were that (1) the charge recombination kinetics were highly nonexponential and (2) the state $D^+Q_AQ_B^-$ could be completely (≥98%) regenerated after the initial charge separation was allowed to recover. The recombination kinetics after this regeneration were identical with those found after the initial illumination.

Temperature Dependence of the Recombination Kinetics. Samples were cooled to 77 K under illumination and then brought to the temperature at which the kinetics were to be determined. The charge separation was regenerated by a brief illumination and the recombination kinetics of $D^+Q_AQ_B^-$ measured. The results for different temperatures are shown in Figure 4. As in the case of RCs with one quinone, the kinetics remained unchanged and reversible for temperatures below ~90 K. Above 90 K the kinetics changed with time; they were no longer reversible as the temperature was cycled[3] and the ability to regenerate the state $D^+Q_AQ_B^-$ became partially lost.

Electron Transfer from $D^+Q_A^-Q_B$ to $D^+Q_AQ_B^-$ at 77 K. The electron transfer time τ_{AB} was determined at room temperature from the kinetics of the optical absorbance changes characteristic of the oxidation states of the two quinones (Vermeglio & Clayton, 1977). These absorbance changes are relatively small; therefore, the results of many flashes must be averaged. At cryogenic temperatures the long lifetime of $D^+Q_AQ_B^-$ makes signal averaging not practical. We, therefore, used different methods to estimate the transfer time, τ_{AB}, for RCs cooled under illumination and in the dark.

The method used for RCs cooled under illumination (assuming that eq 3 is still valid) is based on the following considerations: Absorption of light leads to the formation of $D^+Q_A^-Q_B$. The electron on Q_A^- can either recombine directly with D^+ or pass to Q_B to form $D^+Q_AQ_B^-$. When the lifetime of the state $D^+Q_AQ_B^-$ is long compared with the other transfer times (i.e., τ_{BD}, $\tau_{BA} \gg \tau_{AD}$, τ_{AB}), the fraction $[D^+]/[D^+]_{max}$ measured after a laser flash (more precisely after a time $t^{-1} \simeq \tau_{AB}^{-1} + \tau_{AD}^{-1}$) is given by the ratio $\tau_{AD}/(\tau_{AB} + \tau_{AD})$ (Chamorovsky et al., 1976; Kleinfeld et al., 1984). Since τ_{AD} is known, the value of τ_{AB} can be determined from this ratio.

The above method was used to estimate τ_{AB}^{light} in RCs cooled to 77 K under illumination. After the state $D^+Q_AQ_B^-$ was allowed to decay at 77 K in the dark for 10^5 s, the sample was illuminated by a laser flash and $[D^+]$ was determined from the absorption change at 890 nm. The value of $[D^+]_{max}$ was found from the absorption change under continuous illumination. The extent of $[D^+]$ measured after a laser flash was found to be within 2% of the maximum value (i.e., $[D^+]/[D^+]_{max} \geq 0.98$). With $\tau_{AD} \simeq 10^{-1}$ s, this result leads to an estimate[4] of $\tau_{AB}^{light} < 10^{-3}$ s.

When RCs were cooled in the dark to 77 K and illuminated with a laser flash, D^+ decayed to zero (i.e., $[D^+]/[D^+]_{max} \rightarrow 0$) with a time (25 ms) characteristic of τ_{AD}^{dark}. This result leads to the conclusion that $\tau_{AB}^{dark} \gg \tau_{AD}^{dark}$, i.e., $\tau_{AB}^{dark} \gg 2.5 \times 10^{-2}$ s, and therefore, $\tau_{AB}^{dark} \gg \tau_{AB}^{light}$.

In an alternate method, we attempted to place a longer limit on τ_{AB}^{dark} by monitoring the accumulation of D^+ under continuous, weak (nonsaturating) illumination. Since $\tau_{AB}^{dark} \gg \tau_{AD}^{dark}$ (see above), the states DQ_AQ_B and $D^+Q_A^-Q_B$ are in equilibrium on the time scale with which electrons leave Q_A^- to form $D^+Q_AQ_B^-$. If, in addition, we make the ad hoc assumption that the lifetime of $D^+Q_AQ_B^-$ is long compared to the transfer time from Q_A^- to Q_B (i.e., τ_{BD}^{dark}, $\tau_{BA}^{dark} \gg \tau_{AB}^{dark}$), the electrons will accumulate on Q_B^- with a time constant of τ_{AB}^{dark}. This accumulation will cause an increase in the optically monitored concentration of D^+. It can be shown from eq 3 that, to first order in time, the change in $[D^+]$ is given by

$$\frac{[D^+(t)]}{[D^+(0)]} = \frac{\Delta A^{890}(t)}{\Delta A^{890}(0)} = 1 + \left(\frac{\tau_{AD}^{dark}}{\tau_{h\nu} + \tau_{AD}^{dark}}\right)\frac{t}{\tau_{AB}^{dark}} \quad (4)$$

where $[D^+(0)]$ and $\Delta A^{890}(0)$ are the concentration and absorbance when the illumination is turned on (more precisely, after a time $t^{-1} \simeq \tau_{h\nu}^{-1} + \tau_{AD}^{-1}$).

The experiment was performed at 77 K with the light intensity adjusted to make $\tau_{h\nu} = \tau_{AD}^{dark}$ (half-saturation). After 6×10^3 s of continuous illumination, we found less than a 2%

[3] This caused the data at 111 K presented earlier (Kleinfeld et al., 1983) to deviate slightly from the one in this paper.

[4] This estimate is applicable only to the fraction (~60%; see Figure 4) of RCs that had recovered within 10^5 s.

change in $\Delta A^{890}(t)$. By use of eq 4, this results in $\tau_{AB}^{dark} > 10^5$ s, i.e., 8 orders of magnitude longer than τ_{AB}^{light}. It should, however, be kept in mind that this result will only be valid if our assumption pertaining to the lifetime of the $D^+Q_AQ_B^-$ state for RCs cooled in the dark is correct.

Theoretical Model

In this section we present a model to explain the deviation of the charge recombination kinetics from a simple exponential. The formalism used is analogous to that developed by Austin et al. (1975) for the binding of CO to myoglobin.

The charge recombination kinetics depends on the wave functions and energy levels of the acceptor and donor and the shape (e.g., height and width) of the energy barrier between them. From a structural point of view, the parameters that affect the recombination kinetics are the distance between the donor and acceptor, their relative orientation, and the electronic structure of the intervening medium. If all the donor–acceptor pairs had identical parameters, the recombination kinetics would follow an exponential decay; i.e., the observed absorption change $\Delta A(t)/\Delta A(0)$ would be given by

$$\Delta A(t)/\Delta A(0) = e^{-t/\tau} \tag{5}$$

where τ is the characteristic recombination time. A deviation from this simple expression can be formally described by a distribution in τ, as has been done by Ke and co-workers to explain the recombination kinetics in photosystem I of green plants (Ke et al., 1979). We wish to relate the distribution of τ to a structural parameter. For simplicity we shall consider only variations in the electron-transfer distance, r, between the donor and acceptor. It should be kept in mind, however, that in reality any of the other parameters mentioned above (e.g., orientation and barrier height) could also be distributed. The recombination time will depend on the overlap between the donor and acceptor state wave functions. If one assumes that these wave functions decrease exponentially in amplitude with increasing distance, $\tau(r)$ follows the relation

$$\tau(r) = \tau(\bar{r})\, e^{(r-\bar{r})/r_0} \tag{6}$$

where r_0 is a scaling factor of the order of 1 Å (Hopfield, 1974; Jortner, 1976), whose exact value depends on the details of the system, and $\bar{r}$ is the average distance of the electron-transfer path between the reactants.[5] For the D–Q_A pair, $\bar{r}$ has been estimated to be ~20 Å (Redi & Hopfield, 1980; Jortner, 1980).

If r varies between different donor–acceptor pairs, we can describe it by a normalized distribution function $\mathcal{D}(r)$ (i.e., $\int_0^\infty \mathcal{D}(r)\, dr = 1$), and eq 5 becomes

$$\Delta A(t)/\Delta A(0) = \int_0^\infty \mathcal{D}(r)\, e^{-t/\tau(r)}\, dr \tag{7}$$

We wish to solve eq 7 for $\mathcal{D}(r)$ using the measured values of $\Delta A(t)/\Delta A(0)$. Changing the variables of integration (eq 6), i.e., $dr = -\tau(r) r_0 d[1/\tau(r)]$, and defining

$$\mathcal{P}[1/\tau(r)] = r_0\, \tau(r)\, \mathcal{D}(r) \tag{8}$$

eq 7 becomes

$$\Delta A(t)/\Delta A(0) = \int_0^{1/\tau(0)} \mathcal{P}[1/\tau(r)] e^{-t/\tau(r)}\, d[1/\tau(r)] \tag{9}$$

where the upper limit ($r = 0$) is obtained from eq 6:

$$1/\tau(0) = [1/\tau(\bar{r})] e^{\bar{r}/r_0} \tag{10}$$

As will be shown in the next section, $1/\tau(0)$ can be taken as infinity. The distribution function $\mathcal{P}[1/\tau(r)]$ in eq 9 is then given by the inverse Laplace transform:

$$\mathcal{P}[1/\tau(r)] = \mathcal{L}^{-1}\{\Delta A(t)/\Delta A(0)\} \tag{11}$$

By use of the functional dependence of $\Delta A(t)/\Delta A(0)$ given by eq 2, the above expression can be solved (Abramowitz & Stegen, 1965) for the distribution functions,[6] that is

$$r_0\mathcal{D}(r) = \frac{\mathcal{P}[1/\tau(r)]}{\tau(r)} = \frac{1}{\Gamma(n)}\left[n\frac{\tau_0}{\tau(r)} \right]^n e^{-n[\tau_0/\tau(r)]} \tag{12}$$

where $\Gamma(n)$ is the gamma function.

Calculation of the Distribution of Distances from the Observed Kinetics

The distribution of donor–acceptor electron-transfer distances is described by the function $r_0\mathcal{D}(r)$, given by eq 12, in which τ_0 and n are experimentally determined parameters and $\tau(r)$ is given by eq 6. We shall first treat the case of RCs with one quinone, i.e., the charge recombination kinetics $D^+Q_A^- \rightarrow DQ_A$. All distances will be related to the electron-transfer distance in reaction centers cooled in the dark (r_{AD}^{dark}). Since for this case the decay kinetics closely approximates an exponential, we can write

$$\tau(\bar{r}_{AD}) = \tau_0^{dark} = 22 \text{ ms} \qquad \bar{r}_{AD} = \bar{r}_{AD}^{dark} \tag{13}$$

Note that for a pure exponential decay, $\tau_0^{dark} = \tau_{AD}^{dark}$. For RCs coded in the dark, the difference between the two time constants is small, i.e., 22 vs. 25 ms.

We use eq 13 to justify the replacement of the upper limit in the integral of eq 9 by ∞. With $\tau(\bar{r}_{AD}) = 22$ ms, $\bar{r}_{AD} = 20$ Å, and $r_0 = 1$ Å, the limit (eq 10) $1/\tau(0) \simeq 10^{10}$ s^{-1}. This is much larger than the inverse time scale involved in the measured kinetic changes (see origin of abscissa in Figure 2) and thus justifies the replacement of $1/\tau(0)$ by ∞.

The calculated distributions of distances (using the values of n at τ_0 of Figure 2) for RCs cooled in the dark and cooled under illumination are shown in Figure 5. The two main features of the distributions are the following: (1) the average electron-transfer distance in RCs cooled under illumination ($\bar{r}_{AD}^{light}$) is larger by $1.1r_0$ (i.e., ~1 Å) than the average distance in RCs cooled in the dark ($\bar{r}_{AD}^{dark}$); this represents an ~5% increase in r_{AD}; (2) the width of the distribution in RCs cooled under illumination is ~2.5 larger than it is in RCs cooled in the dark.

It should be noted that the distribution function $r_0\mathcal{D}(r)$ is only meaningful for those states that have decayed within the time of the measurement, t_{max}. These correspond to RCs that have an electron-transfer distance up to a maximum value given by eq 6, that is

$$[(r_{AD} - \bar{r}_{AD})/r_0]_{max} = ln\, [t_{max}/\tau(\bar{r}_{AD})] \tag{14}$$

For the data in Figure 2, $(r_{AD} - \bar{r}_{AD})/r_0 = 1.9$ and 5.2 for RCs cooled in the dark and under illumination, respectively. These values occur far in the tails of the distributions (see Figure 5). Thus, the experimentally observed absorption changes assayed essentially the entire population of RCs.

We now turn to the charge recombination kinetics $D^+Q_AQ_B^- \rightarrow DQ_AQ_B$ observed in RCs with two quinones. The observed

[5] This distance is not necessarily the minimum, i.e., edge-to-edge, distance between the reactants. The exact pathway of the electron will depend on the electronic structure of the intervening medium.

[6] An alternate way to characterize $\mathcal{P}[1/\tau(r)]$ is by its moments; these are related to the derivatives of the experimental data with respect to time [see, for example, Kittel (1958)]. This characterization can be accomplished without assuming a functional dependence of the measured values of $\Delta A(t)/\Delta A(0)$. However, this procedure is very sensitive to noise in the data, and the results are difficult to visualize.

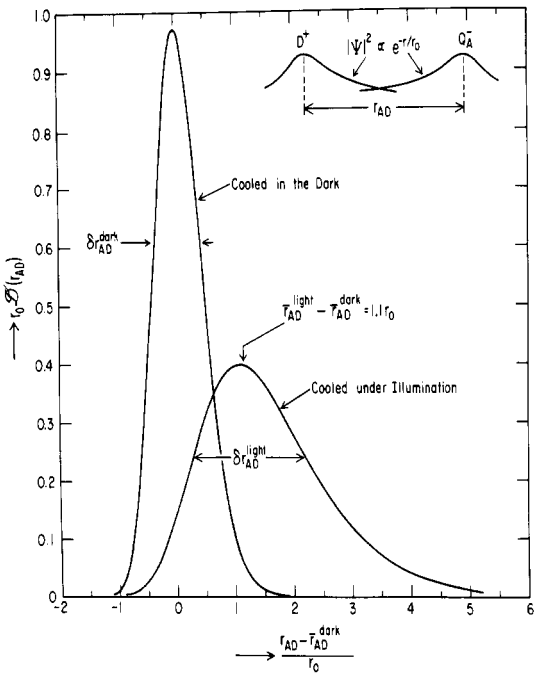


FIGURE 5: Calculated distributions (see eq 12) of the electron transfer between D^+ and Q_A^- in 1UQ/RC samples cooled in the dark and under illumination. This distribution describes the nonexponential decay kinetics of $D^+Q_A^-$, shown in Figures 1 and 2. The experimental parameters, n and τ_0, given in Figure 2, were used together with eq 6 and 12 to calculate the distributions. Insert shows the exponential decrease of the wavefunctions that leads to eq 6. Note that RCs cooled under illumination have a larger average electron-transfer distance as well as a larger spread in distances than RCs cooled in the dark.

kinetics are also well described by a power law (eq 2), as can seen from the good fit of the data in Figure 4. However, for this case the complete relation between $\tau(r)$ and r (analogous to eq 6) is not known; consequently $\mathcal{D}(r)$ cannot be obtained. The distribution $\mathcal{P}(1/\tau)$ could be calculated, but it would be highly truncated in view of the limited data recorded (i.e., at 77 K, the maximum temperature at which reversible kinetics were measured, only ~50% of the charges had recombined up to t_{max}).

Discussion and Conclusions

We have shown that the kinetic properties of RCs cooled to cryogenic temperatures under illumination (i.e., in the charged separated state) differ from those cooled in the dark.

For RCs containing *one quinone* we modeled the observed kinetics of charge recombination in terms of a distribution of structural configurations, specifically donor–acceptor electron-transfer distances. For RCs cooled under illumination, the distribution broadened and shifted to larger distances. The shift and width of the distribution was of the order of 1 Å, which is similar to the root mean square displacements determined from crystallographic studies on other proteins (Frauenfelder et al., 1979; Artymiuk et al., 1979) and from model calculations (Karplus & McCammon, 1981). The recombination kinetics were essentially temperature independent between 4.2 and 77 K, indicating that the distribution remained constant with temperature on the time scale of the measurements.

It is interesting to speculate whether the light-induced changes might have a physiological function analogous to those produced by allosteric changes in other systems. The key feature of the charge separation process in photosynthesis is the high quantum yield, brought about by the slow charge recombination as compared with the fast forward transport of the electrons along the transfer chain. Perhaps the structural changes accompanying the charge separation process act to inhibit the wasteful direct recombination pathway. The possibility of such a stabilization process has been discussed by Warshel (1980) and by Woodbury & Parson (1984).

For RCs containing *two quinones* the recombination kinetics, τ_{BD}, were found to be strongly temperature dependent, the decay at 18 K slowing down for the majority of RCs to $\tau_{BD} \gg 10^5$ s (see Figure 4). If, in analogy with the kinetics of charge recombination of $D^+Q_A^-$, the recombination kinetics of $D^+Q_AQ_B^-$ is intrinsically temperature independent, the observed temperature dependence can be explained by the model of Agmon & Hopfield (1983a). Due to the dynamics of protein motion, the RC passes through a number of structural states; the most favorable states for rapid recombination are those for which the distances between Q_B^- and D^+ are small. As the temperature is raised, the probability that transitions to these favorable states occur is increased, thereby reducing the recombination time τ_{BD}. This interpretation requires that, at cryogenic temperatures, transitions between structural states occur on the time scale $t \lesssim 10^3$ s (see Figure 4). For RCs with one quinone, the recombination time, τ_{AD}, is much shorter ($\sim 10^{-1}$ s) than this time scale. Consequently, there is no opportunity to sample the different conformational states within the time τ_{AD}. This gives rise to an effective static distribution of distances between Q_A^- and D^+ resulting in temperature-independent kinetics as observed in RCs with one quinone.

The above ideas can be extended to explain the exponential behavior found for τ_{AD} at room temperature. If the structural states are sampled in a time $t \ll \tau_{AD}$, the individual states will not be expressed and a single, average, τ_{AD} will be observed. This situation is analogous to the case of motional narrowing in magnetic resonance [see, for example, Pake & Estle (1973)] and is supported by similar findings for ligand binding to heme compounds (Austin et al., 1975; Agmon & Hopfield, 1983b). Thus, under physiological conditions, the transitions between structural states probably occur on a time scale much shorter than 10^{-1} s.

The trapping of D^+ at cryogenic temperatures for an essentially infinite time was reported by McElroy et al. (1974). They suggested that the trapping was caused by a conformation change. We believe that this is not the case but is the result of the loss of an electron from Q_A^- (or Q_B^-) before cooling. This loss occurs as a consequence of a lengthy period of illumination (McElroy et al., 1974) ($t > 10$ s) at room temperature. There is, presumably, an exogenous acceptor (of unknown origin) present that can be reduced by Q_A^- (or Q_B^-).

We now turn to the light-induced changes of the electron transfer from Q_A^- to Q_B. For RCs cooled in the dark, this transfer was not observed. This is to be expected, in view of the large enthalpy of activation for this process (Mancino et al., 1984; Kleinfeld et al., 1984). However, when RCs were cooled under illumination, the transfer time was $\tau_{AB} < 10^{-3}$ s. Thus, the height of the activation barrier decreases dramatically when RCs are cooled in the $D^+Q_AQ_B^-$ state. One possible mechanism for this change is the binding of a proton near Q_B^-. It has been shown that electron transfer from Q_A^- to Q_B at room temperature is energetically favorable only if Q_B^- associates with a proton (Kleinfeld et al., 1984). This proton is likely to be trapped near Q_B^- after the RCs are cooled and may be responsible for the increase in transfer rate.

Acknowledgments

We thank E. C. Abresch for isolating the reaction centers,

D. B. Pendergraft for assistance with the measurements, and J. P. Allen, D. R. Fredkin, and E. M. Gullikson for many useful discussions.

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