Kinetics of electron transfer in duroquinone-reconstituted reaction centers from photosynthetic bacteria

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1. INTRODUCTION

Reaction centers isolated from photosynthetic bacteria are of interest both as a model system for biochemical electron transfer and as a simple system for study of the primary photochemistry of photosynthesis. The light-induced electron-transfer processes in purple photosynthetic bacteria begin with excitation of a bacteriochlorophyll (Bchl) dimer with maximum absorbance at 870 nm (P870). A relatively stable charge-separated state, consisting of oxidized P870 and reduced ubiquinone (UQ), is reached in < 1 ns [1,2]. An earlier unstable state almost certainly consists of $P870^+$ and reduced bacteriopheophytin (BPh) [3]. Ubiquinone extraction leads to an apparent loss of photochemical activity than can be recovered by addition of UQ or a variety of other quinones [4–7]. Here, we report experiments on reaction centers reconstituted with duroquinone (DQ, 2,3,5,6-tetramethylbenzoquinone). We have found that the quantum yield of formation of the state $P870^+DQ^-$ is essentially 1.0. The temperature dependence of the recombination kinetics of this state are anomalous, with temperature-independent regions at both low and high temperatures.

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Abbreviations: Bchl, bacteriochlorophyll; BPh, bacteriopheophytin; RC, reaction center; UQ, ubiquinone; DQ, duroquinone; LDAO, lauryl dimethylamine oxide; EDTA, ethylenediamine tetraacetic acid

2. MATERIALS AND METHODS

Reaction centers (RCs) from *Rhodopseudomonas sphaeroides* R-26 were isolated using the detergent lauryl dimethylamine oxide (LDAO), essentially as in [8]. UQ extraction was accomplished using the methods in [5]. As measured by the apparent loss of photochemical activity at 870 nm, all UQ was extracted from 85–90% of the RCs. Reconstitution of UQ-depleted RCs was effected by incubation in varying concentrations of DQ. Essentially identical results were obtained using LDAO concentrations from 0.02–1% LDAO.

Photochemical activity was measured at 865 nm using a homemade single-beam kinetic spectrophotometer consisting of a Candela SLL625 flashlamp-pumped dye laser, a Nicolet Explorer III digital oscilloscope, and a Hewlett-Packard 9825 T computer. The detection system consisted of a tungsten–halogen lamp, monochromators, and a United Detector Technology 455 Si photodiode/amplifier.

Transmittance changes induced by single 583 nm laser flashes were stored in the transient recorder, transferred to the computer, and numerically converted to absorbance changes (ΔA). First-order decay constants were determined using a linear least squares fit of $\ln \Delta A$ *vs* time. In DQ-reconstituted RCs, contributions from RCs containing residual UQ were eliminated by analyzing only the latter portion of the decay curves. Analysis was begun only after $P870^+UQ^-$ recombination was $>95\%$ complete.

Binding constant determinations were done

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using two different methods. In the first, RCs were incubated in varying concentrations of DQ at 22°C for 2 h. Small corrections (< 5%) were made in the free DQ concentrations to account for DQ bound to RCs. The second method involved the use of homemade plexiglass microdialysis cells, in which depleted RCs were dialyzed against solutions of known DQ concentration until a constant level of photoactivity was observed. This method eliminates any ambiguities due to non-specific binding.

Variable temperature experiments were performed using an unsilvered Dewar and a 1/8 in. pathlength aluminum and plexiglass cuvette. Stable temperatures of 90–310 K were obtained by passing compressed nitrogen via copper tubing through a liquid nitrogen bath and a heating element to the Dewar. The temperature of the sample was measured with a copper–constantan thermocouple immersed directly in the sample.

Absolute photochemical quantum yields were measured using a flash saturation technique (H.M. Cho, R.E.B., in preparation). Briefly, a laser flash-induced light saturation curve for photobleaching at 870 nm was measured and the entire curve was fitted using a non-linear least squares computer program to an exponential function. Absolute flash energies were measured using a Scientech 360001 joulemeter. This quantum yield method is relatively independent of the recombination rate of the system, an important consideration in experiments such as these where widely different recombination rates are observed.

3. RESULTS AND DISCUSSION

Fig.1 shows representative laser flash-induced transmittance changes in (A) control, (B) UQ-depleted and (C) DQ-reconstituted RCs. The control sample (fig.1A) shows some biphasic decay, reflecting the presence of a second UQ acceptor molecule in ~20% of the RCs. The UQ-depleted sample exhibits strictly monophasic kinetics (k = 10.5 s^{-1}), indicating that all of the second UQ as well as most of the first UQ has been removed (fig.1B). Reconstitution with DQ restores almost 90% of the photochemical activity lost upon extraction (fig.1C). Biphasic kinetics in this sample result from reaction centers containing residual UQ. The $P870^+DQ^-$ state recombines with a first-order decay constant (k = 2.3 s^{-1}) that is independent of both DQ and RC concentration (not shown). The strictly monophasic nature of this decay indicates that all DQ-containing RCs have nearly identical DQ binding sites. This homogeneous behavior was observed at >90 min after reconstitution. At earlier times, $\ln\Delta A$ *vs* t plots showed a slight curvature, indicative of some heterogeneous RC/DQ binding. The $t_{1/2}$ for the changeover from slightly heterogeneous to strictly homogeneous behavior was ~20 min, as measured by the gradual increase in r, the linear correlation coefficient of the $\ln\Delta A$ *vs* t plot, and k, the apparent first-order recombination rate constant. During this period, k increased by ~50%, eventually becoming constant.

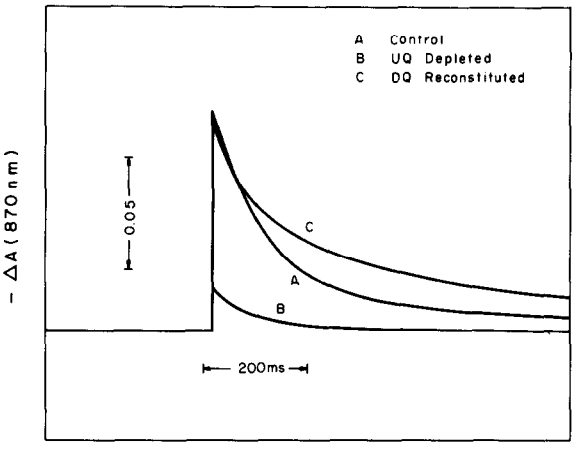

Fig.1. Laser flash-induced absorbance changes measured at 870 nm: (A) untreated RCs; (B) UQ-depleted RCs, k = 10.5 s^{-1}; (C) DQ-reconstituted RCs, k = 2.32 s^{-1}. For all samples, RC was 1.85 μM in 10 mM Tris (pH 8.0), 0.1% LDAO, and 10 μM EDTA buffer. An upward deflection is a transmittance increase and absorbance decrease.

Dissociation constant data are shown in fig.2. Both the simple incubation and equilibrium dialysis techniques yielded an apparent dissociation constant of DQ from reconstituted RCs of 2×10^{-5} M. Little effect of detergent concentration (0.02–1%) was observed. This K_d is considerably larger that of 1×10^{-7} reported in [6]; we have been unable to resolve this discrepancy. Although the dissociation constant for UQ has not been precisely determined, it is undoubtedly in the nM range. The much tighter binding of UQ is probably facilitated by its long hydrophobic tail.

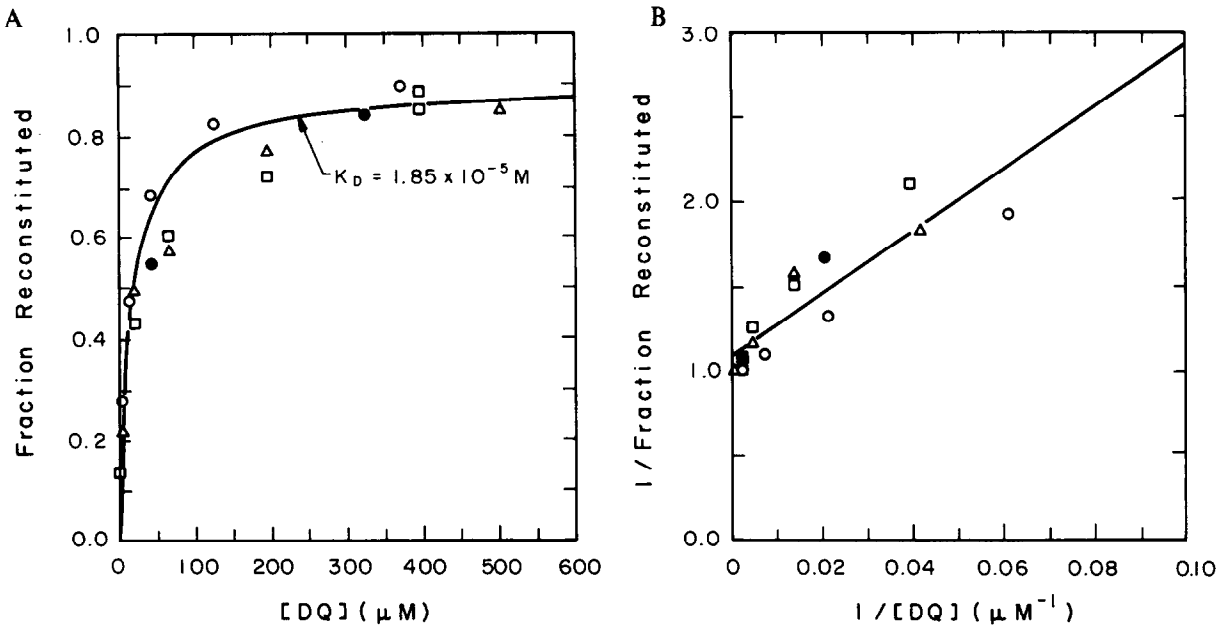

Fig.2. Binding of duroquinone to UQ-depleted RCs: (A) simple saturation curve; (B) double reciprocal plot; (△) 1.88 μM RC LDAO = 1.0%; (○) 1.80 μM RC, LDAO = 1.0%; (●) 3.18 μM RC, LDAO = 1.0%; all measured by simple incubation; (□) 1.13 μM RC, LDAO = 0.01%, measured by equilibrium dialysis. The solid line in both plots is a theoretical plot assuming K_d = 1.85 × 10^{-5} M and 90% possible reconstitution. It is the linear least squares fit to the data of (B).

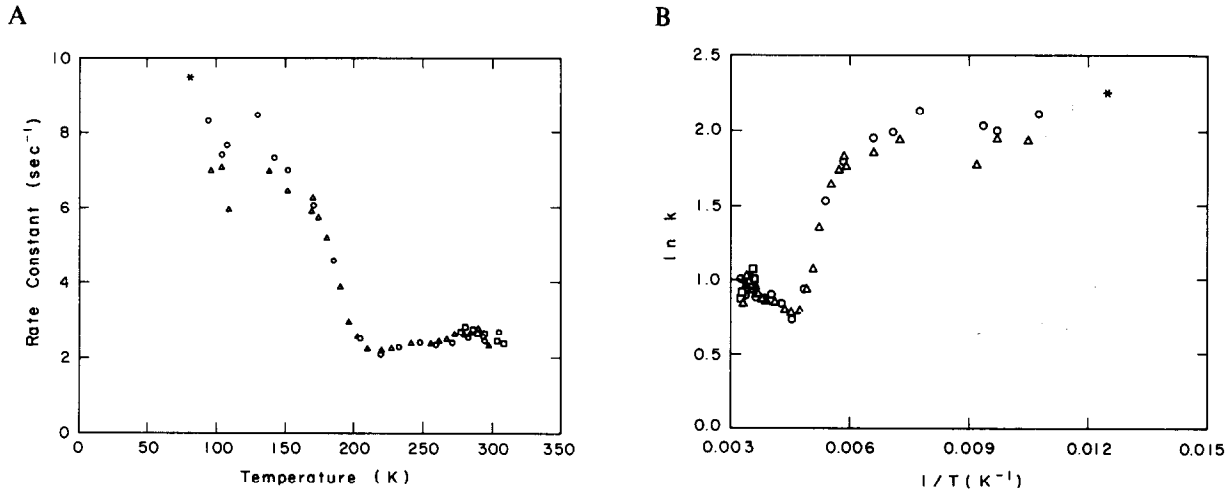

Fig.3. Temperature dependence of P870+DQ^- decay constant: (A) direct plot of *k vs T*; (B) is an Arrhenius plot; (△) 1.96 μM RC, 196 μM DQ; (○) 2.45 μM RC, 230 μM DQ; both in 75% (v/v) glycerin; (□) 3.44 μM RC, 344 μM DQ in 12.5% ethylene glycol; (*) from [5].

The temperature dependence of the $P870^+DQ^-$ $\rightarrow$P870DQ recombination reaction is shown in fig.3. Temperature independence was observed at < 125 K. From 125–200 K an inverse temperature dependence with an apparent activation energy of -2.4 kcal/mol was observed (fig.3B). At > 200 K, the recombination rate constant exhibited a very slight normal activation energy of $+0.3$ kcal/mol. RCs containing residual UQ exhibited no significant temperature dependence of k at < 125 K, and an inverse temperature dependence with an apparent activation energy of -0.6 kcal/mol at higher temperatures. No discontinuity was observed in the 200 K region in UQ-containing samples (not shown).

The temperature independence of the decay of $P870^+DQ^-$ in the low temperature region is evidence for direct recombination, thought to occur via nuclear tunneling [9–12]. The inverse temperature dependences suggest that both the $P870^+DQ^-$ $\rightarrow$P870DQ and $P870^+UQ^-$ $\rightarrow$P870UQ reactions occur in the 'activationless' region of the rate *vs* free energy curve [10–13]. The change to nearly temperature-independent decay in the high-temperature region in DQ-containing samples is surprising. Nuclear tunneling is expected only at low temperatures, where all vibrations have settled into the ground vibrational level, and not at elevated temperatures, where a distribution of vibrational states is found. There are a number of possible explanations for this behavior:

(1) The simplest explanation is that this is indeed the intrinsic behavior of the recombination reaction. Although most theories of electron transfer do not predict such behavior, the possibility of temperature dependence of this sort was suggested in [14].

(2) A reversible conformational change of the RCs could be occurring at ~ 200 K, perhaps in response to solvent phase changes. This also could reflect greater solvent accessibility to the quinone site in DQ-containing RCs. Evidence for such effects has been observed in a variety of systems [15], but UQ-containing RCs show no discontinuity in decay rate at this temperature.

(3) In addition to the direct recombination, $P870^+DQ^-$ recombination can occur by a competing pathway with a normal activation energy. At low temperatures such a pathway would be frozen out, and only direct recombination could occur. Recombination via the $P870^+BPh^-$ state is possible, but the ~ 800 mV free energy gap between the $P870^+BPh^-$ state and the $P870^+DQ^-$ state predicts a much stronger temperature dependence than that observed [16]. The in situ redox potential of DQ is not known, but the in vitro polarographic half-wave reduction potentials of DQ and UQ are very similar [17], so we expect the in situ potentials also to be similar. We thus feel it unlikely that this pathway is responsible for the observed behavior.

Recombination may occur via an as yet undetected state intermediate in energy between $P870^+BPh^-$ and $P870^+DQ^-$. This decay pathway may well exist in UQ-containing samples as well, but the faster direct recombination between $P870^+$ and UQ^- precludes any substantial contribution by this pathway at physiological temperatures. A similar effect at higher temperatures was observed in UQ-containing RCs from *Rps. sphaeroides* [18], supporting this idea.

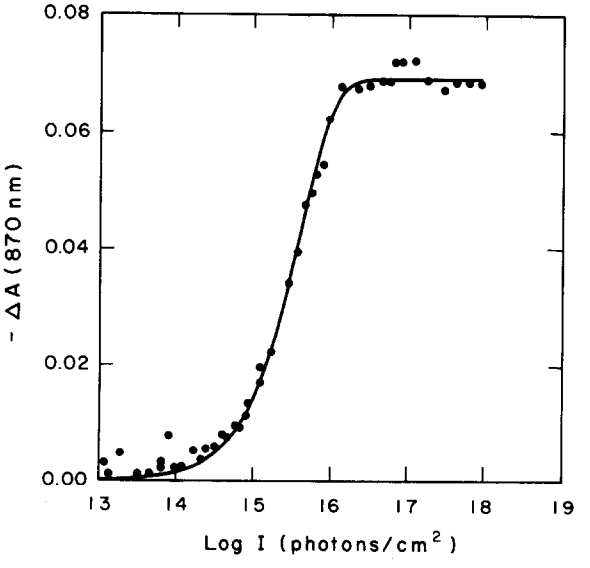

Fig.4. Measurement of the quantum yield of formation of state $P870^+DQ^-$. The solid line is a theoretical plot for quantum yield $\Phi = 1.12$, generated by a non-linear least squares fitting routine.

Fig.4 shows the results of a flash-induced measurement of the absolute quantum yield of formation of the $P870^+DQ^-$ state. The method measures the quantum yield of formation of only the state actually being observed, in this case $P870^+DQ^-$, and is not distorted by contributions from RCs with residual UQ. An exponential fit (solid line in fig.4) to the data yields an absolute quantum yield $\Phi = 1.12$, while control RCs measured in a parallel experiment gave $\Phi = 1.05$. Slight systematic errors in the measurements of the RC extinction coefficient and the absolute flash energy are undoubtedly responsible for the values being slightly greater than unity. However, it is clear that the quantum yields of $P870^+Q^-$ formation in the two samples are nearly identical ($\Phi_{DQ}/\Phi_{UQ} = 1.07$). The quantum yield for UQ-containing systems has been measured to be essentially 1.0 [19,20]. This result that the rate constant for the $P870^+BPh^-DQ \rightarrow P870^+BPh\,DQ^-$ reaction must be $> 10^9\ s^{-1}$ [21]. A slower rate constant for this reaction would be manifested as a decrease in Φ for formation of state $P870^+\,DQ^-$, since a $10^8\ s^{-1}$ competing pathway for $P870^+BPh^-$ recombination is present [3].

We have shown that DQ reconstitutes stable charge separation with a high quantum yield in UQ-depleted reaction centers. The temperature dependence of the recombination is unusual and may reflect the presence of a hitherto undetected state in the reaction center electron-transfer pathway.

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