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Effect of quinone depletion on lifetime spectra in photosynthetic reaction centers

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It is shown that a well defined inhomogeneity consisting in a partial loss of the primary quinone (Q) may give rise to a spectral dependence of the lifetime of the radical pair state P^+H^-, P denoting the primary donor and H bacteriopheophytin. The lifetime spectrum follows from a complex decay pattern of H^- due to the forward electron transfer on the 100 ps time scale and the recombination dynamics of the radical pair P^+H^- which is by two orders of magnitude slower. Since the underlying biexponential decays are detectable only in measurements with high sensitivity including sufficiently long delay times, the complex kinetics might be easily hidden in a single-exponential fitting function. Nevertheless, a simulation for 20% Q-depletion demonstrates, that at room temperature the monoexponentially fitted rate for $P^+H^- \rightarrow P^+Q^-$ may vary by a factor of ≈ 2 when probed at wavelengths between 786 and 812 nm. Thus, a sensitive determination of the occupancy of the primary quinone site is necessary when lifetime spectra in the 100 ps range are observed.

1. Introduction

Although in recent years detailed structural [1–3] and kinetic information has become available for photosynthetic reaction centers (RCs), many questions concerning the microscopic description of electron transfer processes are still open. One of the most important problems relates to the significance of kinetic data since the role of inhomogeneities of structure or energetics is not yet understood. Such inhomogeneities, as e.g. a distribution of protein conformations, might manifest themselves in lifetime spectra of intermediates [4]. It is the goal of this paper, to demonstrate that also a different class of inhomogeneities arising from a partial loss of a cofactor may lead to a spectral dependence of transient decays. Such an inhomogeneity may be realized in preparations of native RCs which tend to loose some primary quinone (Q) during the isolation procedure.

The loss of Q in a small fraction of RCs may lead to an apparent spectral dependence of the rate k_Q for electron transfer from the reduced bacteriopheophytin (H) to Q occurring on the 100 ps time scale,

$$P^+H^-Q \rightarrow P^+HQ^- , \tag{1}$$

where P^+ denotes the cation of the primary donor. In the fraction of Q-depleted RCs the state P^+H^- is long-lived (≈ 10 ns) and decays via spin-dependent recombination reactions according to

$$P^+H^- \rightarrow PH, {}^3P^* H \tag{2}$$

to both, the ground state PH and the triplet state $^3P^*$. As a necessary consequence, absolute quantum yield measurements performed on a time scale > 10 ns are expected to yield values smaller than unity. Although the decay times in (1) and (2) differ by two orders of magnitude, the concomitant prevalence of some other conditions (to be specified below) may lead to a complex phenomenology by superposition of both processes and thereby to a dependence of the monoexponentially fitted rate k_Q on the wavelength of probing.

The analysis presented in this paper has been initiated by the experimental observations that the standard preparation of RCs from *Rb. sphaeroides* shows an average loss of 20% Q, when LDAO is used as detergent in the absence of excess quinone [5].

The extent of Q-depletion can be controlled in an unambiguous diagnostic method which measures the recovery dynamics of the absorption of the primary donor (P) over the large time window between 1 ns and 500 ms [5]. Even after reconstitution with ubiquinone-10 still 10 ± 3% Q were found to be missing. In general, the loss of Q tends to be more pronounced in modified RCs, irrespective whether the modification was induced by mutagenic replacements of amino acid residues in the protein framework [6,7] or by replacements of cofactors in a thermal exchange procedure [8].

2. Wavelength-dependent absorption transients in the 100 ps time domain

2.1. Principle of data analysis

Any treatment of a spectrally dependent decay pattern rests on the assumption of a superposition of two difference absorption bands related to different species decaying with the time constants τ_1 and τ_2. Depending on the relative magnitudes of τ_1 and τ_2 the following situations can be met:

(a) If $\tau_1 \approx \tau_2$ holds, deviations in a monoexponential fit will be small and the resulting rate is expected to reflect an amplitude-weighted, average value.

(b) For $\tau_1 < \tau_2$, a biexponential decay should be detectable.

(c) In the case $\tau_1 \ll \tau_2$, the slow component is often treated as a time-independent off-set underlying a monoexponential fit of the fast decay in a time window $t_f \ll \tau_2$. However, for specific relations between a given time window of the fit, lifetimes and amplitudes, the validity of a monoexponential fitting procedure may break down, thereby giving rise to artefacts, as e.g. spectrally dependent rates. As we will show, such a breakdown of a usually justified approximation may be difficult to recognize.

2.2. Simulations

The effect of a small fraction of Q-depleted RCs on the spectral dependence of decay rates is tested in the spectral region around 800 nm. At short times, ≈ 10 ps, and long times, ≈ 5 ns, the predominant feature of the difference spectrum in this region is caused by an electrochromic shift of the Q_y-absorption band of the bacteriochlorophyll monomer. After short times the difference spectrum is independent of the presence of Q, whereas after long times according to our model different contributions may play a role:

(i) Within 200 ps the monomer absorption band is shifted to the red following process (1).

(ii) This red-shift is superimposed on a difference spectrum originating from the fraction of Q-depleted RCs, which at this time have not yet recombined. This fraction gives rise to a difference spectrum similar to the 10 ps spectrum, but characterized by amplitudes decaying within the lifetime τ_{RP} of the radical pair P^+H^-.

Thus, neglecting for the sake of simplicity the fast formation kinetics of P^+H^-Q and P^+H^-, the kinetics underlying the spectral changes in fig. 1 can be described for an arbitrary fraction D of Q-depleted RCs by the temporal evolution of the absorbance $A(t)$,

$$A(t) = (1-D)\{\Delta\epsilon_{P^+H^-Q}\exp(-t/\tau) + \Delta\epsilon_{P^+HQ^-}[1-\exp(-t/\tau)]\} + D\Delta\epsilon_{P^+H^-}\exp(-t/\tau_{RP}), \qquad (3)$$

where $\Delta\epsilon$ refers to the relative difference absorption coefficients of the subscript states and the lifetimes τ and τ_{RP} are related to P^+H^-Q and P^+H^-. The first term accounts for RCs containing Q, the second term

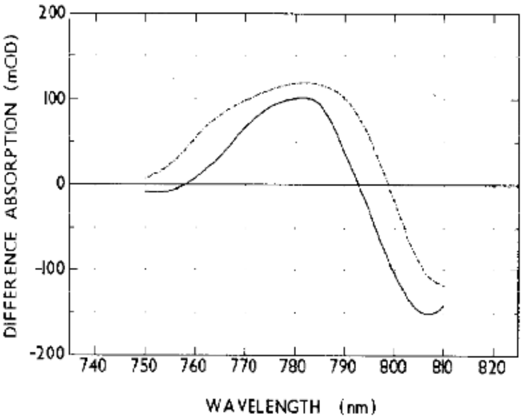

Fig. 1. Schematic drawing of transient absorption spectra at 285 K at 11.4 ps (—) and 2.7 ns (–·–·–) after excitation of RCs of *Rb. sphaeroides* (after ref. [4]).

for the Q-depleted ones. The treatment in (3) is further simplified by the (well justified) assumption that in the spectral region of fig. 1 contributions from the triplet state $^3P^*$ formed in the recombination process (2) are small and may therefore be neglected.

Using the model function (3) biexponential decays in the spectral window between 750 and 812 nm are simulated taking for $\tau=210$ ps [9], $\tau_{RP}=13$ ns [10] at 285 K and $\tau=130$ ps [9], $\tau_{RP}=23$ ns [10,11] at 80 K. The relative difference extinction coefficients were extracted from typical short time and long time spectra for RCs of *Rb. sphaeroides*. An example is given in fig. 1 [4,12]. The extraction of $\Delta\epsilon$ from difference spectra which possibly relate to 100% Q-content is justified since the wavelength dependence of $\Delta\epsilon$ is rather insensitive to the actual Q-content of 80% (see the appendix). Simulations are performed in the time window 0–3000 ps with a resolution of 20, 50 and 200 ps in the respective intervals, 0–200, 200–1000, 1000–3000 ps. Thus, by imposing two fixed time constants an "artificial" decay pattern for different wavelengths has been created.

If we now fit our simulated biexponential decay curves in a monoexponential way, neglecting radical pair recombination in the 20% fraction of Q-depleted RCs, we indeed obtain wavelength-dependent decay times as shown. They increase from 186 ps at 786 nm to 404 ps at 812 nm. In the blue edge of the difference band we envisage a smaller increase from 192 ps at 770 nm to 216 ps at 750 nm. In table 1 these monoexponential, wavelength-dependent decay times are compiled together with the maximal deviations R between the biexponential calculated decay curves and their monoexponential fits. Since R obtained for the time range 0–3000 ps is of the order of 2.5 mOD, the influence of Q-depletion can only be resolved in experiments with highest accuracy.

3. Results and discussion

Based on the values in table 1 and the analogous low temperature data, the lifetime spectra of P^+H^-Q shown in fig. 2 have been constructed for 285 and 80 K. At room temperature, a 20%-depletion of Q leads to an appreciable effect on the monoexponential lifetime, especially in the red wing of the spectrum, whereas this effect almost disappears at low temperatures. Thus, the Q-content has to be controlled whenever lifetime spectra are measured. Or alternatively, biexponentiality of the decay has to be tested in time windows which are sufficiently large.

The simulated lifetime spectra are indeed rather surprising in view of the large discrepancy in the two lifetimes τ and τ_{RP} (by a factor of ≈ 100) and the small fraction of Q-depleted RCs ($\approx 20\%$) underlying the simulation. The origin of this pronounced effect is due to a complex interplay of the fitting window ($t_f < \tau_{RP}$) and ratio of the two lifetimes as well as the relative wavelength-dependent amplitudes by which both processes (1) and (2) contribute to the decay curve.

Even under the condition that only 20% of the RCs

Table 1

Spectral dependence of decay times at 285 K obtained in monoexponential fits of biexponential decay curves. Maximal deviations between these fits and the simulated curves are given by the value R. By reducing (expanding) the time window for the fit, the variation of the monoexponential decay times is expected to decrease (increase)

λ (nm)	τ (ps)	R (mOD)	λ (nm)	τ (ps)	R (mOD)
750	216	0.15	786	186	1.60
754	213	0.16	790	205	0.60
758	211	0.05	794	212	0.35
762	207	0.30	798	219	1.40
766	203	0.70	802	228	2.20
770	192	1.20	806	250	2.50
			808	265	2.40
			810	313	2.00
			812	404	1.60

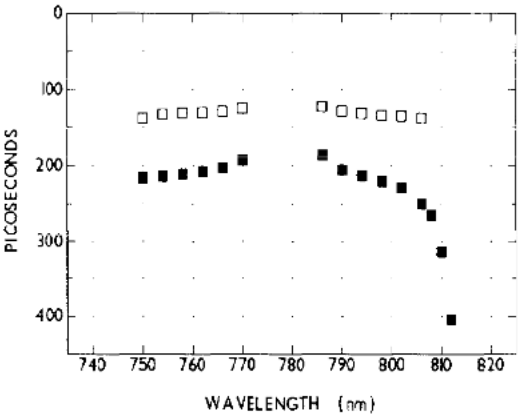

Fig. 2. Lifetime spectrum obtained from monoexponential fits of biexponential simulated decay curves at 285 K (full squares) and 80 K (open squares). The reduction of the spectral range in the low temperature simulation is due to the negligible amplitude originating from process (1). The "artificial" spectrum is based on relative difference absorption coefficients derived from ref. [4], the lifetimes $\tau=210$ ps (285 K); $\tau=130$ ps (80 K); $\tau_{RP}=13$ ns (285 K); $\tau_{RP}=23$ ns (80 K), pertaining to ≈20% Q-depleted RCs.

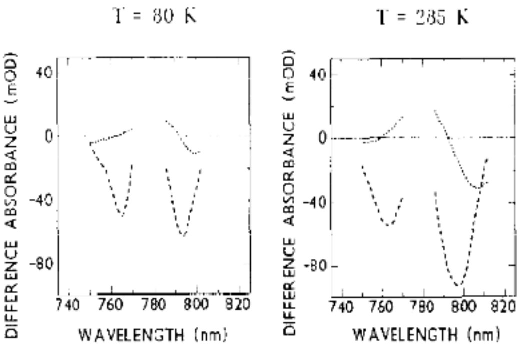

Fig. 3. Absolute amplitudes of signals representing the decay of P^+H^-Q (– – –) with the relative amount $1-D$ and P^+H^+ (···) with the relative amount D at high and low temperatures.

are Q-depleted, the amplitudes of the two components, given by $(1-D)(\Delta\epsilon_{P^+H^-Q}-\Delta\epsilon_{P^+HQ^-})$ and $D\Delta\epsilon_{P^+H^-}$ can become comparable by the following antagonism:

(i) Reaction (1) occurring in the majority of RCs may lead to relatively small amplitudes for the decay of P^+H^-Q in regions where the difference extinction coefficients of P^+H^-Q and P^+HQ^- may become quite similar as for example at $\lambda=808$ nm (fig. 3 at $T=285$ K).

(ii) The recombination of P^+H^-, although occurring in a minority of RCs, may then result in a large relative contribution to the decay signal. The spectral dependence of this amplitude follows essentially the 11.4 ps spectrum reduced in its amplitude by the fraction D (fig. 3). Since the amplitude of this component changes sign as a function of wavelength, the monoexponentially fitted decay times can change in both directions: they increase (decrease) in spectral regions where the sign of both contributions is the same (opposite).

In principle, the contribution of the slow recombination process in Q-depleted RCs could be reduced by (i) fitting in a shorter time window, however, at the expense of accuracy, or (ii) in a biexponential fit using a long time window, provided the accuracy of the data set allows for it.

In the model proposed, the wavelength-dependent kinetics should tend to disappear in two cases:

(1) Losses in Q are negligible as to be expected in RCs of *R. viridis* where the occupancy of the Q-binding site is reported to be 1.0∓0.1 menaquinone/RC [13].

(2) The ratio τ_{RP}/τ is large or the corresponding ratio of amplitudes is small. Indeed, the increase of $\tau_{RP}/\tau=85$ at 285 K by a factor of 3 upon cooling to 80 K turns out to be the predominant factor leading to the disappearance of the wavelength dependence of the decay at 80 K (fig. 2). Moreover, this effect is amplified by a decrease of the ratio of the amplitudes of the relevant radical pair states, P^+H^- and P^+H^-Q (fig. 3) under the condition of the well known spectral shifts at low temperatures.

Some trends of the simulated lifetime spectrum (fig. 2) are also encountered in experiments [4]; e.g. the increasing lifetime in the red wing of the spectrum towards longer wavelengths as well as the disappearance of this effect at low temperatures. The wavelength dependence of the formation process of P^+H^-, which was also reported [4], cannot be accounted for in terms of our model, since this primary charge separation occurs on ultrafast time scale of 3–4 ps at 285 K [14,15].

Details of the influence of Q-depletion on spectrally dependent decay rates are studied in extensive simulations and in experiments accompanied by a highly sensitive independent control of the relative

Q-content [5]. The effect is expected to occur also in other regions of the difference spectrum and is observed at present in sensitive pump–probe experiments in the range 520–660 nm (standard deviation of ±0.4 mOD) [9]. Contributions of this effect to lifetime spectra are expected in native and modified RCs in which the reduction of Q is inhibited, either by its absence or by conformational changes as e.g. those revealed after cooling during illumination [16].

4. Conclusions

Wavelength-dependent electron transfer kinetics in the 100 ps range representing the time domain of reduction of primary quinone in the reaction center shows a variation of the rate in monoexponential fits of biexponential simulations. These simulations refer to probing absorbance in the spectral window between 750 and 812 nm at 285 K and show that the effect is expected to disappear upon lowering the temperature, e.g. at 80 K. When basing the simulations on a 20% depletion of primary quinone a maximum variation of the apparent reduction rate ($P^+H^- \rightarrow P^+Q^-$) by a factor of ≈ 2 is obtained for room temperature. The resolution of the underlying biexponential decay pattern due to the dual decay channels, $P^+H^- \rightarrow P^+Q^-$ and $P^+H^- \rightarrow PH$, $^3P^*H$, is only possible when probing with high sensitivity up to long delay times. Thus, whenever lifetime spectra are observed in the 100 ps time domain, especially around 800 nm, a sensitive and independent control of the occupancy of the binding site of the primary quinone is necessary before conclusions on other sources of inhomogeneities can be drawn, as e.g. a distribution of different protein conformations.

From a more general point of view, the simulations performed in this paper demonstrate how the complex and hard-to-quantify phenomenon of lifetime spectra from protein bound pigments can originate from a well defined inhomogeneity, i.e. a partial depletion of one of the cofactors. In the case considered, a small fraction of the primary quinone is assumed to be lost. Since quinones can be reconstituted, this hypothesis may be put to a parametric test in a straightforward way.

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Appendix. Extraction of difference extinction coefficients

In the frame of our model function (4) the relative difference extinction coefficients are given by the amplitudes A_1 and A_2 of the difference spectra (285 K: fig. 1 [4], 77 K: fig. 1 [12]) at the times $t_1 = 11.4$ ps and $t_2 = 2.7$ ns. Assuming $\Delta\epsilon_{P^+H^-Q} = \Delta\epsilon_{P^+H^-}$ we obtain

$$\Delta\epsilon_{P^+H^-} = \Delta\epsilon_{P^+H^-Q} = k_1/k_2 ,$$

$$\Delta\epsilon_{P^+HQ^-} = \frac{A_2 - D\exp(-t_2/\tau_{RP})\,\Delta\epsilon_{P^+H^-}}{1-D} ,$$

with

$$k_1 = A_1 - A_2[1 - \exp(-t_1/\tau)]$$

and

$$k_2 = (1-D)\exp(-t_1/\tau) + D - D[1-\exp(-t_1/\tau)]\exp(-t_2/\tau_{RP}) .$$

The amplitudes thus obtained for 285 K are at $\lambda = 786$ nm: +88, +115 mOD; $\lambda = 810$ nm: −140, −117 mOD; $\lambda = 750$ nm: −8, +8 mOD and $\lambda = 770$ nm: +68; +99 mOD after 11.4 ps and 2.7 ns, respectively. Difference extinction coefficients extracted with the assumption of zero Q-depletion are hardly affecting the lifetime spectra.

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