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The electron transfer rate from BPh_A^- to Q_A in reaction centers of *Rhodobacter sphaeroides* R-26: Influence of the H-subunit, the Q_A and Fe^{2+} cofactors, and the isoprene tail of Q_A

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The secondary electron transfer from reduced bacteriopheophytin (BPh_A^-) to the first acceptor quinone (Q_A) in variously modified reaction centers from *Rhodobacter sphaeroides* was studied by transient absorption spectroscopy and compared with native reaction centers. In intact reaction centers, neither substitution of the native Q_A, ubiquinone-10 (UQ_{10}), with menaquinone (MK), nor shortening of the isoprene tail of ubiquinone (UQ) down to 4 and that of MK down to 2 isoprene units changed the rate of electron transfer to Q_A. However, in Fe^{2+}-depleted reaction centers the electron transfer rate decreased by a factor of 5 upon MK-reconstitution and by a factor of 20–50 upon non-native UQ-reconstitution. In the latter particles, the electron transfer rate decreased with decreasing tail length of UQ, suggesting that the displacement of UQ within the Q_A pocket as proposed in previous work (Liu B.-L., Van Kan P.J.M. and Hoff A.J. (1991) FEBS Lett. 289, 23–28), is tail-length-dependent.

Introduction

The reaction centers (RC) of the purple bacteria *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides* have been crystallized and their structure has been determined by X-ray diffraction experiments to a resolution of < 3 Å [1–4]. In spite of the tremendous gain in structural knowledge, understanding of electron transfer between the cofactors is still far from complete. In this report we address the influence of the iron-ion and the H-subunit on electron transfer between reduced bacteriopheophytin, BPh_A^- (I^-), and the primary quinone electron acceptor (Q_A), and the relation between quinone structure and isoprene tail length on this process.

The reaction center of *Rb. sphaeroides* R-26 consists of three protein subunits: L, M and H. The cofactors in this RC are 4 bacteriochlorophyll (BChl) *a* molecules, 2 bacteriopheophytin (BPh) *a* molecules, 2 quinones (Q_A and Q_B) and a non-heme high-spin Fe^{2+} ion [5]. The organic cofactors are arranged in two chains of approximate C_2 symmetry, A and B, of which only chain A is photoactive. The L- and M-subunits both bind 2 BChls, 1 BPh and 1 quinone (Q_A to the M-, Q_B to the L-subunit). Q_A in the native RC is ubiquinone-10 (UQ_{10}), the isoprene tail of which is directed towards and well beyond BPh_A (bound to the L-subunit), the distance from Q_A to BPh_A being 3 to 4 isoprene units; Q_B is also a UQ_{10} molecule. Both quinones are magnetically coupled to the iron ion, which is situated between Q_A and Q_B, somewhat closer to the latter [3]. Two BChls, one of the L-subunit and one of the M-subunit, form a dimer, called the special pair or the primary donor (P). Upon excitation by light P is converted to its lowest excited singlet state, P*. Due to this excitation P loses an electron and the radical pair state $P^+BPh_A^-$ (P^+I^-) is created within about 3 ps [6,7]. The exact pathway of this primary charge separation is still a subject of discussion. In approx. 200 ps (a characteristic first order time-constant) the electron is transferred from I^- to Q_A [7–9], and in about 150 μs from Q_A^- to Q_B [7,10]. If Q_A is prereduced or absent, P^+I^- recombines to form either the singlet ground or the triplet state of P in about 10–12 ns [7,11].

Secondary electron transfer from I^- to Q_A has been the subject of many investigations [7,11–20]. The influence of the H-subunit and of the Q_A and Fe^{2+} cofac-

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Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin; I, intermediary electron acceptor; MK, menaquinone; P, primary electron donor; Q_A, primary quinone electron acceptor; RC, reaction center; UQ, ubiquinone.

tors on this electron transfer has been investigated in different laboratories with contradictory results [7,12, 13].

The effect of H-subunit depletion of the RC on the functioning of the RC was examined by Debus et al. [13]. They observed no change in photochemical activity, i.e., the formation of $P^+Q_A^-$, upon the removal of the H-subunit. They also concluded that the H-subunit has little or no effect on the binding site of Q_A. Experiments by Liu et al. [12] confirmed the conclusions of Debus et al. about the primary photochemical activity. However, they observed in addition that in RCs without Fe^{2+}, which were not investigated by Debus et al., the presence of the H-subunit does affect secondary electron transfer.

Much work has also been done on the characterization of electron transfer in RCs that had been depleted of the native Q_A and reconstituted with different quinone species [11,14–16]. Picosecond measurements of the decay of the absorption of I^- showed that quinones with in situ redox potentials (E_m) different of that of the native Q_A exhibit a slower electron transfer from I^- to Q_A^- than native RCs, e.g. for anthraquinone (AQ) (E_m = −0.16 V) and methylbenzoquinone (E_m = +0.07 V) the transfer times are ~ 1 ns [11,14] and 400 ps [11], respectively. For RCs reconstituted with AQ derivatives the quantum yield of the $P^+Q_A^-$ formation was less than unity, implying that forward electron transfer was so slow that recombination processes can compete with it [15]. Gunner et al. [16] concluded from quantum yield measurements of $P^+Q_A^-$ formation that the length of the isoprene tail of UQ has no effect on the secondary electron transfer. They also reported a dependence of secondary electron transfer on the free energy difference ($-\Delta G^0$), which is a measure for the redox potential in situ, between the states $P^+I^-Q_A$ and $P^+IQ_A^-$. They observed that the quantum yield of $P^+Q_A^-$ formation was at least 0.9 for RCs reconstituted with quinones that had a $-\Delta G^0$ between −150 meV and 50 meV relative to the native UQ. Lowering $-\Delta G^0$ to more than −150 meV relative to UQ, the quantum yield of $P^+Q_A^-$ formation dropped significantly. This suggests that in the latter case charge recombination competes with secondary electron transfer.

Investigations by Blankenship and Parson [17] and Agalidis et al. [18] showed that Fe^{2+} depletion of the RC did not change the rate of electron transfer from I^- to Q_A^-. The former authors probably also removed the H subunit from the RC as discussed by Liu et al. [12]. Kirmaier et al. [19], however, reported that Fe^{2+} depletion of the RC slowed down the decay of the radical pair state P^+I^- by a factor of ~ 20 to 4.2 ± 0.3 ns, and that the quantum yield of $P^+Q_A^-$ formation dropped from 1.0 to 0.47. They concluded that the iron does play a significant role in the electron transfer from I^- to Q_A^-.

Recently, Liu et al. [12,20] reported similar results as found by Kirmaier et al. In addition, they found that the time constant of the secondary electron transfer in RCs depleted of the H-subunit and in RCs depleted of both Fe^{2+} and the H-subunit was ~ 200 ps, i.e., essentially the same as in native RCs. They concluded that both Fe^{2+} and the H-subunit play an important role in the secondary electron transfer and a model was proposed in which Q_A can occupy two sites in the RC, the possibility of two binding sites for Q_A in the RC of *Rb. sphaeroides* being indicated by Allen et al. [4]. Liu et al. [12] suggested that in Fe^{2+}-depleted RCs the less favorable of these two sites is occupied by Q_A and that this causes a less efficient electron transfer from I^- to Q_A^-.

Some of the conclusions about the electron transfer time mentioned above are based on measurements of the quantum yield of $P^+Q_A^-$ formation. This technique, however, is not informative for quantum yields close to unity (> 0.9), i.e., when the rate of electron transfer to Q_A is much larger than the rate of the recombination reactions of the radical pair P^+I^-. In this case direct measurement of the I^- absorption decay kinetics is a more sensitive method to discriminate between the effects of modifications of the RC on the electron transfer time and will therefore provide a better insight in the transfer mechanism.

In this work we present measurements of the kinetics of electron transfer from BPh_A^- (I^-) to Q_A in various modified RCs. Preparations were used with the following RC compositions: intact RCs, LM-complexes (LM), Fe^{2+}-depleted RCs (RC/dFe) and Fe^{2+}-depleted LM-complexes (LM/dFe). These (modified) RCs were reconstituted with ubiquinones (UQ) or menaquinones (MK) of which the length of the isoprene tail was varied from 2 up to 10 isoprene units. This is the first time the interrelation of the influence of the Q_A and Fe^{2+} cofactors and the H-subunit on the secondary electron transfer rate is examined systematically.

We found that electron transfer in the MK-reconstituted RCs was as fast as in the UQ-reconstituted RCs. Only a small effect of the length of the isoprene tail on the electron transfer is observed in intact RCs. In RC/dFe complexes reconstituted with UQs we observe a strong dependence of the electron transfer rate on the length of the isoprene tail of UQ. The kinetics of the RC/dFe preparations are in agreement with both Kirmaier et al. [19] and Liu et al. [12] and our results support the model proposed by the latter.

Materials and Methods

LDAO was purchased from Fluka Chemical Corporation; UQ_{10}, UQ_9, UQ_7, UQ_6, UQ_4, BSA and DL-dithiothreitol were from Sigma; MK_8, MK_6, MK_5, MK_4,

MK_3 and MK_2 were generous gifts from Hoffmann La Roche, Switzerland; sodium cholate, sodium deoxycholate, EDTA, KSCN and 1,10-*o*-phenanthroline monohydrate were from Merck; DE52 was from Whatman Biosystems Ltd.; $LiClO_4$ was from BDH Chemicals Ltd..

Q_A-reconstitution solution. All quinones were used for reconstitution without further purification. They were dissolved in a sodium deoxycholate solution by the procedure previously described [20].

Q_A-depleted reaction centers. Native reaction centers from *Rb. sphaeroides* R-26, prepared as described elsewhere [12], were depleted up to 96% of both Q_A and Q_B and subsequently reconstituted with various quinones (Q_A) by the method of Liu et al. [20]. For a few RC preparations Q_A removal and reconstitution was done according to Ref. 21.

Q_A-depleted, Fe-depleted and Q_A-reconstituted reaction centers. The removal of Fe^{2+} and the reconstitution of the quinone was performed in a single step as described in Ref. 20, except that other quinones instead of UQ_{10} were used for reconstitution and a modified procedure of Tiede and Dutton [22] instead of the procedure of Debus et al. [13] was followed for the removal of the Fe^{2+}. Thus, in a typical procedure, Q_A-depleted reaction centers in 10 mM Tris-HCl, 0.05% LDAO (pH 8.0) mixed with the Q_A-reconstitution solution were dialyzed against 1 M KSCN, 10 mM Tris-HCl, 0.05% LDAO, pH 8.0 at room temperature for 1–2 h and then dialyzed at 4°C for 20 h against 10 mM Tris-HCl, 0.05% LDAO, 0.1 mM EDTA, pH 8.0, followed by purification on a column of DE52. They were subsequently eluted with 0.5 M NaCl in 10 mM Tris-HCl, 0.05% LDAO (pH 8.0) and finally dialyzed against 10 mM Tris-HCl, 0.1% sodium cholate, 0.1 mM EDTA (pH 7.7) at 4°C for 1 day.

Fe^{2+}-depleted, H-depleted and Q_A-reconstituted reaction centers. LM-complex without Fe^{2+} (LM/dFe) was prepared as previously described [12], except that other quinones instead of UQ_{10} were used for the reconstitution of quinone. LM complex with Fe^{2+} (LM) was similarly prepared, except that no *o*-phenanthroline was added to the dialysis solution.

Q_A content after reconstitution. The Q_A content of all preparations was determined by measuring the photochemical activity as described elsewhere [20].

Fe^{2+} content of LM / dFes and RC / dFes. The Fe^{2+} content of the LM/dFes and the RC/dFes was measured by means of atomic absorption spectroscopy as previously described [20]. Both types of preparation contained < 15% Fe^{2+} with respect to native RCs.

Decay kinetics of P^+I^-. Absorbance differences due to the BPh_A^- anion were measured at 668 nm using a pump-probe method. For excitation we used the frequency-doubled 532 nm pulse of a passively mode-locked Nd:YAG laser (JK Lasers). The duration of the excitation pulse was ~ 25 ps. A weak probe pulse was generated by passing the remaining 1064 nm fundamental pulse through a cuvette filled with an H_2O/D_2O mixture. From the resulting continuum pulse the desired probe pulse was extracted by means of a small monochromator with a bandwidth of about 1 nm. The instrumental response, resulting from the cross-correlation of pump and probe pulses, was approximated by a gaussian of 35 ps fwhm. Fits to the measured kinetics were convolutions of exponential decays with this instrument response function. The experimental error was estimated from the results for several preparations measured under various conditions. The error in picosecond decay times was 10 ps, in ~ 1 ns decay times 0.1 ns, and in nanoseconds decay times 0.5 ns. The samples were measured in a cuvette of 1 mm pathlength and had an A_{865} of about 20 cm^{-1}. During the experiments the samples were cooled to a temperature between 0 and 2°C.

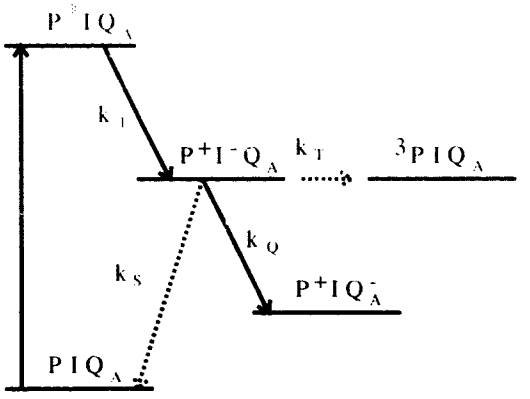

Fig. 1. Possible electron transfer pathways within the first 5 ns after excitation of the bacterial reaction center.

Results

The electron transfer reactions that take place in the bacterial RC on the time scale of our measurements (0–5 ns) are shown in Fig. 1. The excitation of P and subsequent charge separation with rate constant $k_1 = 3.6 \cdot 10^{11}\ s^{-1}$ is too fast to be observable with our equipment. The secondary electron transfer rate constant, k_Q, can be determined accurately by measuring the decay of the absorption of I^- at 668 nm, which reflects the depopulation of the state $P^+I^-Q_A$ by recombination to the singlet ground or triplet state (rate constants k_S and k_T, respectively) and forward electron transfer from I^- to Q_A. These are three competing decay channels and the analysis of the I^- absorption decay will therefore give a rate constant k given by:

$$k = k_Q + k_{rec} \qquad (1)$$

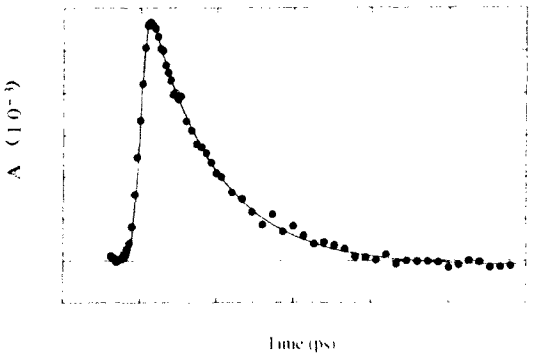

Fig. 2. The decay of I^- absorption measured at 668 nm of RCs reconstituted with UQ_6. The RCs were excited at 532 nm with a non-saturating laser flash of 0.7 mJ cm^{-2}. The sample had an $A_{800} = 2.5$ mm^{-1} and the temperature was 2 ± 2 C. The decay curve is fitted with a single exponential with decay time of 177 ± 10 ps.

TABLE I

Time constants of forward electron transfer from BPh_A to Q_A determined as described in the text ($\tau = (k_Q)^{-1}$)

Quinone	RC	LM/dFe	RC/dFe
	τ in ps [a]	τ in ps [a]	τ in ns
UQ_1	163	216	7.7 [b]
UQ_2	177	234	
UQ_5	190		6.7 [b]
UQ_6	192	163	4.4 [b]
UQ_{10}	198 [c]		3.6 [b]
MK_3	166	142	0.9 [c]
MK_4	164		
MK_5	148	184	
MK_6	175		
MK_7	179	188	1.0 [c]
MK_8		175	

Experimental errors [a] ± 10 ps, [b] ± 0.5 ns and [c] ± 0.1 ns. [c] Native reaction center

where the recombination rate $k_{rec} \approx k_S + k_T$. The recombination processes of P^+I^- are governed by the radical pair mechanism, which is a coherent process not easily described by a simple rate constant. Schenck et al. [23] and Chidsey et al. [24] determined the rate of decay of P^+I^- in RCs where Q_A had been removed. They found that, despite of the complexity of the reaction pathway, the overall decay could be described by a single exponential rate constant, although this is most likely not the case for the individual processes leading to the formation of the singlet and triplet state, respectively. For the work presented here this rate constant, k_{rec}, will be set equal to 10^8 s^{-1} as reported by Logunov et al. [11]. The value of k_{rec} will affect the results obtained for k_Q when k_Q is of the same order of magnitude as or smaller than k_{rec}, but in general we found that $k_Q > k_{rec}$ (*vide infra*). Therefore, the error introduced will be small and unidirectional, and will not influence the qualitative conclusions of this work.

In the quinone-reconstituted RCs, LM complexes and LM/dFe complexes only a fast single exponential decay was observed with a relatively small constant component. In these cases the time constant found for the decay is taken to be the reciprocal of the forward electron transfer rate k_Q, since it is much shorter than that associated with charge recombination. A typical result including a fit is given in Fig. 2, which shows the I^- absorption decay of a RC reconstituted with UQ_6.

The time constants of the I^- absorption recovery found in intact RCs reconstituted with a series of UQs and MKs are summarized in the second column of Table I. It is seen that in RCs reconstituted with UQs we find time constants of electron transfer from BPh_A to Q_A ranging from 160 to 200 ps, whereas in intact RCs reconstituted with MKs these time constants vary from 150 to 180 ps. For a few samples it was checked that the two different preparation methods mentioned in Materials and Methods yielded the same decay times within the experimental error. We observe no dramatic changes in the electron transfer rate as a function of the length of the isoprene tail with respect to the native RC; just a small decrease in electron transfer rate is observed with increasing tail length. Our measurements show no significant difference in electron transfer rates in RCs with UQ or MK acting as Q_A.

The measurements performed on LM/dFe complexes reconstituted with UQs and MKs show the same kinetics of the I^- absorption as in quinone-reconstituted RCs. Fig. 3 shows the data and fit for an LM/dFe complex reconstituted with UQ_6; a time constant of 160 ps was found for this preparation. The results for the other LM/dFe preparations are listed in the third column of Table I. The time constants of forward electron transfer in these preparations differ

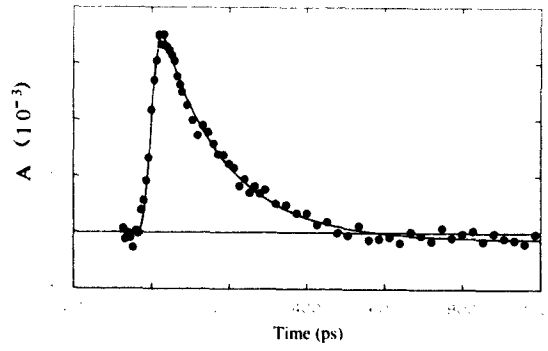

Fig. 3. The decay of I^- absorption measured at 668 nm of LM/dFe-complexes reconstituted with UQ_6. Conditions were as in Fig. 2 except that the excitation energy was 0.5 mJ/cm^2 and $A_{800} = 0.9$ mm^{-1}. A single-exponential fit to the decay curve yielded a decay time of 163 ± 10 ps.

only slightly from the time constants observed in intact RCs; they vary from 160 to 240 ps upon UQ reconstitution and from 140 to 190 ps upon MK reconstitution.

In order to check the dependence of electron transfer from I^- to Q_A on the removal of the H subunit we investigated two LM preparations, one of which had been reconstituted with UQ_6 and the other with UQ_{10}. For these preparations we observed the same kinetics of the I^- absorption decay as in RCs and LM/dFe complexes. The time constants for forward electron transfer were determined to be 160 and 220 ps in LM complexes reconstituted with UQ_6 and UQ_{10}, respectively. These results are very similar to those obtained for intact RCs and LM/dFe complexes.

Analysis of the data obtained from I^- absorption decay measurements in RC/dFe complexes is somewhat more complicated than with the other preparations. First of all, there turned out to be competition between the back reaction and forward electron transport, and the absorbance does not completely recover due to population of the triplet state, which has some absorption at 668 nm. This effect is taken into account by the addition of a second term in the analysis given by:

$$\Delta A_T(1-\exp(-kt)), \qquad (2)$$

where ΔA_T represents the triplet absorption, and k is the rate constant for the decay of the I^- absorption. Secondly, a correction had to be made for the fraction of RCs that had retained Fe^{2+}. These should give rise to a rapid decay occurring in 15% of the RCs. The time constants of this rapid decay were taken from the second column of Table I.

In RC/dFe complexes reconstituted with UQs and MKs we observed a slower decay of the I^- absorption than in intact RCs and LM/dFe complexes. The transfer rate decreased by a factor ~5 and 20–50 upon reconstitution with MK and UQ, respectively. The time constants of forward electron transfer in RC/dFe complexes reconstituted with UQs and MKs were calculated by means of Eqn. 1 and are given in the fourth column of Table 1. Fig. 4 shows the I^- absorption decay of a RC/dFe preparation reconstituted with UQ_9. In RC/dFe complexes containing UQ_{10} we found a decay time of the I^- absorption of ~2.6 ns, somewhat lower than the values reported by Liu et al. [12] and Kirmaier et al. [19], who found ~5 ns and 4.2 ns, respectively. The reason for this difference is not known.

Considering the possibility of an irreversible change of the protein structure upon extraction and reconstitution, we note that the rates of electron transfer in Q_A-reconstituted RCs, LM- and LM/dFe complexes do not significantly differ from the rate of electron transfer in the native RC. Furthermore, experiments by

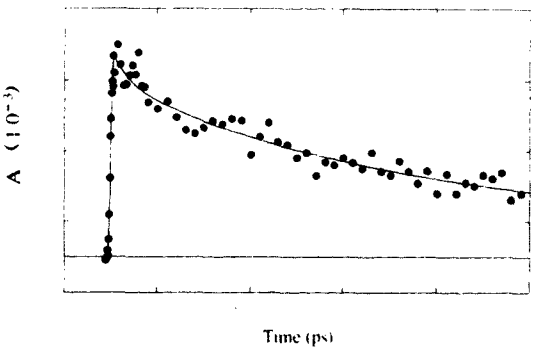

Fig. 4. The decay of I^- absorption measured at 668 nm of RC/dFe-complexes reconstituted with UQ_9. Conditions were as in Fig. 2 except that the excitation energy was 0.4 mJ/cm^2 and $A_{800} = 1.5$ mm^{-1}. The decay of the absorption was fitted with two components: a fast component with a lifetime of 192 ps and a relative amplitude of 0.15, and a slow component, which reflects the sum of the rates of charge recombination and electron transfer, with a lifetime of 3.1 ± 0.5 ns. With $k_{rec} = 10^8$ s^{-1} this yields a lifetime for electron transfer of 4.4 + 0.5 ns.

Liu et al. [12] and Kirmaier et al. [19] demonstrated that reconstitution of Fe^{2+}-depleted RCs with Zn^{2+} almost fully restored the electron transfer. Therefore, we conclude that the observed changes in electron transfer are not caused by irreversible change of the protein structure upon extraction and reconstitution.

Surprisingly, the RC/dFe complexes reconstituted with UQ show a fairly strong dependence of the forward electron transfer on the length of the isoprene tail, which was opposite to that observed in intact RCs. In RC/dFe complexes reconstituted with MKs this feature was not observed and the forward electron transfer was much faster than in UQ-reconstituted RC/dFe complexes.

Discussion

The influence of the H-subunit and the Fe^{2+} cofactor

In this discussion the term electron transfer will refer to the forward electron transfer from BPh_A^- to Q_A unless stated otherwise. The measurements of electron transfer rate in RCs which have been selectively depleted of the H-subunit, the Fe^{2+} ion, or both of these, show that only in RC/dFe complexes is the electron transfer drastically affected. In these complexes we observe a decrease of the electron transfer rate by a factor of 5 upon MK reconstitution and a factor of 20–50 upon UQ reconstitution. These measurements are in agreement with earlier observations [12,19], so far as UQ_{10} reconstituted RC/dFe complexes are concerned. Kirmaier et al. [19] concluded that the Fe^{2+} ion plays an important role in the secondary electron transfer. Liu et al. [12], however, reported that in LM and LM/dFe complexes reconsti-

tuted with UQ_{10} the rate of secondary electron transfer is the same. Our observations on LM and LM/dFe complexes reconstituted with a series of UQs and MKs are consistent with the result of Liu et al. [12], and strongly support their conclusion, that the absence of Fe^{2+} disrupts electron transfer only the in presence of the H-subunit.

The results obtained for LM and LM/dFe complexes by us and Liu et al. also indicate that the H-subunit per se is not essential for fast early electron transfer, in agreement with Debus et al. [13], who studied electron transfer in intact RCs and LM-complexes containing a full complement of Fe^{2+}. However, the H-subunit does affect electron transfer in RCs that have no Fe^{2+}. We conclude that it is hazardous to draw general conclusions on the contribution of Fe^{2+} and the H-subunit to the electron transfer, without examining the interrelationship of these two cofactors

The influence of the Q_A cofactor: UQ vs. MK

Our results show that within experimental error the electron transfer from BPh_A to Q_A is the same for MK- and UQ-reconstituted RCs. At first sight this is rather surprising, as first of all MK has a much lower in vitro redox potential than UQ, viz. −710 and −610 mV in dimethylformamide (DMF), respectively [25], and secondly MK is bulkier than UQ and is expected to fit in a different way in the UQ binding pocket. We will show below, however, that in actuality the effect these differences between the two quinones may have on the electron transfer rates is minor.

The difference in redox potential appears to be much less in vivo than in vitro: $\Delta E_m = E_{MK} - E_{UQ10}$ is −20 mV in situ [15] vs. −100 mV in DMF. Since the quinone redox potential is virtually independent of tail length provided the tail is at least one isoprene unit long [25], we may safely assume that the difference in redox potential for reconstituted MK_{10} compared to the native UQ_{10} is not more than about −20 mV. The influence of this small difference on the electron transfer rate is negligible, as can be seen from the following considerations. Taking the transfer rate at room temperature given by [26]

$$k_{et} = \frac{2\pi}{\hbar}\frac{|V(r)|^2}{\sqrt{4\pi\lambda_A k_B T}}\exp\left[-\frac{(\lambda_A + \Delta G^0)^2}{4\lambda_A k_B T}\right] \qquad (3)$$

with ΔG^0 the difference in free energy of the donor and acceptor states, $V(r)$ the distance-dependent coupling between the donor and acceptor molecules, λ_A the reorganization energy, $\hbar$ Planck's constant, k_B Boltzmann's constant and T the temperature, we can discriminate three plausible cases:

a) λ_A does not depend on the type of quinone, and is midway between $-\Delta G^0_{UQ}$ and $-\Delta G^0_{MK}$. As follows from the parabolic relation (3) the rates are then precisely the same. This possibility, however, is rendered less likely by the observation of Gunner et al. [16] that electron transfer in the native RC is activationless, i.e., $\lambda_A = -\Delta G^0_{UQ}$.

b) λ_A does not depend on the type of quinone, and equals $-\Delta G^0_{UQ}$. For $\Delta G^0_{UQ} = -650$ meV and $\Delta G^0_{MK} = -630$ meV Eqn. 3 then yields $k_{et,UQ} = 1.01\ k_{et,MK}$. This small difference in the rates (~ 2 ps) is well within the margin of experimental error.

c) λ_A depends on the type of quinone, equals $-\Delta G^0_{UQ}$ for the native RC and $-\Delta G^0_{MK}$ for RC reconstituted with menaquinone. Then Eqn. 3 yields $k_{et,UQ}/k_{et,MK} = \exp\left(\lambda_{A,MK}/\lambda_{A,UQ}\right) = 1.006$, again too small a difference to be observable.

The structural difference between the two quinones translates in the possible effects on λ_A discussed above, and in effects on the matrix element $V(r)$ in Eqn. 3, which depends on the distance r as

$$V(r) = V_0 \exp(-\alpha r) \qquad (4)$$

where V_0 is the maximum electronic coupling and $\alpha = -1.6$ Å^{-1} [27]. Our results and those of Ref. 16 show that the tail of the quinones is unimportant for electron transfer (see also below). Therefore, V_0 must result from overlap between the electron clouds of BPh_A and Q_A, likely via superexchange between their π-orbitals and the protein medium. The ring system of MK being twice the size of that of UQ, has twice as many π-electrons as UQ. Increasing the number of π-electrons spreads the relevant molecular orbital (MO) out over more atoms. If the two molecules were fully overlapped face-to-face, this might have little effect on the overall resonance interaction between the two electron-transfer states. If the overlap is less extensive, it could tend to decrease the overall resonance interaction, although this will also depend on the signs and amplitudes of the MO coefficients for the individual atoms. Without detailed structural information about the reconstituted RCs we can not draw firm conclusions about the resonance interaction.

The influence of the larger size of MK compared to UQ on the distance dependence is difficult to estimate. If the protein medium plays an important role as mediator for superexchange interactions, the larger bulk of MK and possibly its somewhat different binding contacts with the protein may not have a large effect, as the amino acids of the protein and the quinones are all in Van der Waals contacts, i.e., r will on the average remain the same, and also the number of contacts is not expected to change very much. Quite likely, there are several pathways for superexchange (see for example [28]) and provided MK does not bind to a site completely different from that of UQ, the net result of MK reconstitution on the electron transfer rate will be neutral.

The situation is different for RC/dFe complexes, where the transfer rate for UQ-reconstituted samples is 4–10 times slower than for MK-reconstituted material. Using Eqn. 4, the slower rate corresponds to a change in distance of 0.7–1.2 Å. Apparently, one or more of the crucial edge-to-edge contacts between UQ and the protein are severely perturbed in the RC/dFe complexes. The change in distance corresponds well with a displacement of UQ in the RC/dFe complexes from the native binding pocket to the less favorable, more distant Q_A site, [12]. This will be further discussed in conjunction with the influence of the tail length of Q_A on electron transfer.

Summarizing, we conclude that for iron-containing RCs it is not difficult to rationalize our observation that the difference in structure and size of MK and UQ is not important for electron transfer, while the small difference in their in situ redox potential translates in a difference in electron transfer rate that is too small to be observable.

Influence of the tail length on the electron transfer

In intact RCs and LM/dFe complexes reconstituted with MKs and UQs with tail length down to 4 units for UQ and down to 2 units for MK we obtained similar electron transfer rates, independent of the length of the isoprene tail. This agrees with quantum yield measurements by Gunner et al. [16] on intact RCs reconstituted with quinones with varying tail length, down to UQ_0. Also in LM complexes reconstituted with UQs with tail lengths down to 6 units and RC/dFe complexes reconstituted with MKs the electron transfer rate is only slightly affected by the tail length. When reconstituted with UQs, however, the latter complexes do show a rather strong dependence of the electron transfer on the tail length.

Before addressing the question why UQ, but not MK, shows a dependence of electron transfer on the length of the isoprene tail in quinone-reconstituted RC/dFe complexes, we will discuss the requirements for the binding of a quinone in the Q_A pocket. The properties of the Q_A binding site have been subject of many investigations [29–33] concentrating on the binding function of both tail and headgroup of the quinone. It has been shown that only the first two isoprene units of the tail contribute to the affinity for the binding site and that a rotational freedom is required between those two units [29–31]. The headgroup seems to be the main contributor to the affinity for the binding site. The size of the ring system of the quinone may greatly influence the affinity; the larger the ring system is, the larger the affinity for the binding site [30,32,33], for example the affinity for MK_0 is one order of magnitude larger than that for UQ_0. For isoprene-tailed MKs and UQs, however, no difference was observed in affinity for the binding site [31]. From these results we conclude that for the quinones that we used for reconstitution in our experiments there is no difference in affinity for the Q_A binding site.

As before, k_{et} can be modified by a change in redox potential, in donor-acceptor distance, in λ_s or in V_{ij}. Liu et al. [12] argued that no change in redox potential of the quinone occurs when Fe^{2+} is removed from the RC. Since intact RCs do not show a tail-length dependence of k_{et}, we may exclude a change of redox potential as the cause of the dependence in RC/dFe preparations. This leaves us with a change in donor-acceptor distance for explaining the tail-length dependence of the electron transfer in UQ-reconstituted RC/dFe complexes.

As mentioned above, the isoprene tail seems to contribute to the stability of the binding of the quinone in the protein matrix. When the length of the tail is decreased, then the position of the quinone will become less stable and it can be moved more easily from its original binding site. We suggest that the absence of the Fe^{2+} allows UQ to be moved away from the Q_A binding site, probably by interaction with the H-subunit [12], and that the tail functions as a counterweight for this movement. For UQ_{10} the new site could be the second Q_A site proposed by Allen et al. [4]. For shorter tail lengths the displacement is larger, and UQ moves further away from the native Q_A site.

For MK-reconstituted RC/dFe the situation is different. The headgroup of MK is somewhat bulkier than that of UQ, so that its displacement induced by Fe^{2+} removal is smaller than for UQ, and its position much less sensitive to variations in the tail length.

Concluding remarks

The results presented in this paper show that only in Fe^{2+}-depleted reaction centers is the electron transfer from BPh_A to Q_A affected, in agreement with previous work [12]. In intact reaction centers, substitution of the native Q_A with MK does not change the electron transfer to Q_A, and the isoprene tail of UQ down to 4 isoprene units and of MK down to 2 units is of no importance for the electron transfer. Furthermore, in Fe^{2+}-depleted reaction centers the electron transfer rate decreases with decreasing length of the isoprene tail of UQ. Following Liu et al. [12] we believe that this decrease originates from a displacement of UQ within the Q_A pocket, which is caused by interaction with the H-subunit. Apparently, the interaction depends on the length of the isoprene tail, which functions as a 'counterweight' to the displacement of UQ.

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