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Functional LH1 antenna complexes influence electron transfer in bacterial photosynthetic reaction centers

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Abstract

The effect of the light harvesting 1 (LH1) antenna complex on the driving force for light-driven electron transfer in the *Rhodobacter sphaeroides* reaction center has been examined. Equilibrium redox titrations show that the presence of the LH1 antenna complex influences the free energy change for the primary electron transfer reaction through an effect on the reduction potential of the primary donor. A lowering of the redox potential of the primary donor due to the presence of the core antenna is consistently observed in a series of reaction center mutants in which the reduction potential of the primary donor was varied over a 130 mV range. Estimates of the magnitude of the change in driving force for charge separation from time-resolved delayed fluorescence measurements in the mutant reaction centers suggest that the mutations exert their effect on the driving force largely through an influence on the redox properties of the primary donor. The results demonstrate that the energetics of light-driven electron transfer in reaction centers are sensitive to the environment of the complex, and provide indirect evidence that the kinetics of electron transfer are modulated by the presence of the LH1 antenna complexes that surround the reaction center in the natural membrane.

Abbreviations: DAD – 2,3,5,6-tetramethylphenylene diamine; H_L – primary electron acceptor in the reaction center; LH1 – light harvesting 1 antenna complex; P – primary donor of electrons in the reaction center; PES – phenazine-ethosulfate; PMS – phenazine-methosulfate; Q_A – primary acceptor quinone; *Rb.* – *Rhodobacter*; RC – reaction center; RC-only – reaction center-only strain; RCLH1 – reaction center plus light harvesting complex 1 strain; TMPD – N,N,N′,N′-tetramethylphenylene diamine

Introduction

Photosynthetic reaction centers (RCs) of plants and bacteria efficiently convert excited state energy into a charge separated state across the photosynthetic membrane. The RC excited state that drives charge separation arises as a result of either direct excitation of RC pigments or energy transfer from the antenna pigments that surround the RC (van Grondelle et al. 1994). The atomic structure of the RCs from two purple non-sulphur bacteria shows that the complex contains two linear, nearly symmetrical branches of redox cofactors that span the photosynthetic membrane (Deisenhofer et al. 1985; Allen et al. 1987; Ermler et al. 1994).

Light-driven electron transfer takes place in an effectively exclusive manner along the branch of redox cofactors most closely associated with the L subunit of the RC (Kirmaier et al. 1985).

In isolated reaction centers, or in the membranes of an antenna-deficient 'RC-only' mutant strain (Jones et al. 1992), transmembrane electron transfer is driven from the first singlet excited state of a pair of excitonically-coupled bacteriochlorophyll molecules that lie close to the periplasmic face of the membrane (the primary electron donor, P). In wild type (WT) RCs, decay of the excited state of the primary donor (P^*) occurs on the timescale of a few picoseconds and results in the formation of an intermediate charge-separated state $P^+H_L^-$, in which an electron resides on the bacteriopheophytin (H_L) in the active (L) branch of pigments and a positive charge is present on P (Kirmaier et al. 1985). It is generally agreed that this so-called primary electron transfer reaction is characterized by a relatively small change in free energy between the reactant (P^*) and product ($P^+H_L^-$) state (the driving force, ΔG°), and a small medium reorganisation energy (λ). In general, the rate of electron transfer is optimal when $-\lambda$ equals the standard free energy change between the donor and acceptor states, P^* and $P^+H_L^-$ in the case of primary electron transfer. Increasing or decreasing ΔG° on either side of this 'balance point' results in a slowing of the rate of electron transfer (Marcus and Sutin 1985).

Several groups have studied the relation between ΔG° and the rate of primary electron transfer in genetically modified RCs in which the driving-force has been altered (Nagarajan et al. 1990, 1993; DiMagno et al. 1992; Jia et al. 1993; Allen and Williams 1995). In principle, such an analysis can be used to obtain a value for the reorganization energy that is associated with primary electron transfer (Bixon et al. 1995). All of these measurements have been carried out on purified RC preparations rather than intact systems in membranes. This has been necessary because measurements of the rate of primary charge separation in the presence of the LH1 antenna are precluded by spectral overlap between the RC and LH1 bacteriochlorophylls, which prevents selective excitation of the RC and promotes detrapping of excitation energy from the RC to the antenna. However, *in vivo* the RC is in intimate contact with the LH1 antenna complex, forming the so-called 'RCLH1' core complex'. With the elucidation of the atomic structure of the peripheral light-harvesting complexes (McDermott et al. 1995) and the availability of a low resolution structure of the core antenna (Karrasch et al. 1995) it has become possible to piece together a complete picture of the photosynthetic unit as it resides in the membrane of purple bacteria (Cogdell et al. 1996). This arrangement raises the intriguing question whether the LH1 antenna has a significant influence on the energetics of the primary electron transfer reaction in RCs *in vivo*, especially since it seems likely that there are excitonic interactions between the chromophores in these complexes (Novoderezhkin and Razjivin 1994; Owen et al. 1997). Furthermore, it has been demonstrated that the membrane environment of the RC effects a small modulation of the rate of primary electron transfer (Beekman et al. 1995), highlighting the sensitivity of the complex to its surroundings.

In this report we have examined whether the LH1 antenna modulates the rate of light-driven electron transfer in the RC, using intracytoplasmic membranes in which the RC is the sole pigment protein complex, and membranes in which the RC is surrounded by the LH1 antenna complex. The midpoint electrochemical potential of the primary donor (E_m P/P^+) in the WT RC and in a series of RC mutants in which the rate of primary electron transfer is modulated by changes at the M210 and L181 positions, has been measured in the presence and absence of the LH1 complex. The driving force for the primary electron transfer is directly related to the midpoint potential of the donor, through the following approximate relationship (DiMagno et al. 1992):

$$\Delta G^\circ = -E_{donor} + E_{acceptor} + E_{coulomb} + G_{P^*} \qquad (1)$$

In which E_{donor} and $E_{acceptor}$ are the midpoint potentials of the donor and acceptor respectively, $E_{coulomb}$ accounts for the change of dielectric constant due to the electron transfer and other effects due to charge interactions and Gp^* is an energy offset for the excited state of the primary donor. Thus in absence of any other changes the driving force is expected to decrease linearly with increasing E_{donor} (i.e. E_m P/P^+). We have also estimated the driving force for primary electron transfer in the different RCs in the presence of the LH1 antenna from time-resolved measurements of the delayed fluorescence that occurs upon chemical prereduction of Q_A. We discuss the possible effects of the LH1 antenna on the energetics of primary electron transfer in the RC.

Table 1. Mid-point redox potential for the P/P^+ redox couple in the RCLH1 and RCO mutants

P/P+ redox potentials (mV)[a]			Time constant for P* decay in RC-only membranes[b]	Bi-exponential fit to fluorescence emission from RCLH1 membranes				
	RCLH1	RC-only	t (ps)	t_1(ns)	A_1(%)	t_2(ns)	A_2(%)	$\chi 2$
YM210 (WT)	467	495	4.8	0.50	98.7	7.56	1.3	1.17
YM210F	487	528	27.7	0.54	96.3	5.70	3.7	1.12
YM210L	493	526	37.9	0.39	96.9	5.21	3.1	1.40
YM210W	512	549	72.5	0.44	92.0	3.32	8.0	1.05
YM210H	427	457	5.8	0.55	99.4	2.44	0.6	0.88
YM210H/FL181H	408	422	4.3	0.70	99.8	4.88	0.2	0.76

[a] The error in the reduction potentials was estimated to be +/– 5 mV except for YM210W(RC-only) where it was +/– 15 mV.
[b] Time constant for decay of the P* excited state in RC-only membranes. Data taken from Beekman et al. (1996).

Materials and methods

Biological material

Measurements were carried out using a set of five site-specific mutants of the *Rb. sphaeroides* RC with replacements of the tyrosine at position M210 and the phenylalanine at position L181 (see Table 1). Details of the construction of the mutant RCs and the genetic system used to express the mutated genes have been given elsewhere (Jones et al. 1992, 1994; Beekman et al. 1996). Strains were used that were devoid of both the LH1 and LH2 antenna complexes (RC-only phenotype), together with strains which lacked only the peripheral LH2 antenna (RCLH1 phenotype) (Jones et al. 1992). Intracytoplasmic membranes were prepared from cells grown under anaerobic conditions in the dark, as described previously (Jones et al. 1992, 1994).

Equilibrium redox tritrations of P

Samples of membranes for redox-titrations were diluted (to A_{860} = 0.3–0.5) in 100 mM Tris buffer (pH 8.0), and the following redox mediators were subsequently added: TMPD (final concentration 20 μM); DAD (70 μM); PMS (20 μM); PES (20 μM). The sample was oxidized by addition of ferricyanide to a final concentration of 1 mM, bringing the solution to a midpoint potential of +500 to +550 mV (*versus* the Standard Hydrogen Electrode) as determined with a calibrated calomel/Pt electrode (O'Reilly et al. 1973). The amount of reduced P at a given redox potential was determined from the flash-induced absorbance change arising from photo-oxidation of P, monitored at either 795 or 810 nm, observed in a laboratory-built single-beam kinetic spectrophotometer with submicrosecond time resolution. Samples were contained in a specially designed redox cuvette with a total volume of 350 μl and an optical path length of approximately 4 mm. The light of a xenon flash lamp (EG&G FX272) with a pulse width of 3 μs ($\sim$22 J/flash) was used to photo-oxidize P, and was guided to the cuvette by means of a quartz fiber-optic lightguide. Decreasing the intensity of the flash by 50% had no effect on the extent of the observed absorbance changes, demonstrating that the flashes were fully saturating. Each measurement consisted of the average result of 16 individual transients, with a 10 s interval between transients to ensure full re-reduction of the sample after each flash. Subsequently the sample was reduced by 10 to 20 mV by adding microliter aliquots of a 100 mM sodium-ascorbate solution. The sample was stirred in the dark for several minutes, and when no further decrease in the ambient potential was observed the kinetic measurements were repeated. In this way a full redox-titration of the P/P^+ couple could be obtained except in the YM210W mutant, which has the highest mid-point potential of the RCs studied and could not be fully oxidized by ferricyanide at the concentrations used. In each case the maximal change in absorbance observed at low potential was taken to represent the 100% reduced state and a standard n = 1 Nernst equation was used to fit the data.

Measurements of delayed fluorescence

Delayed fluorescence in RC/LH1 membranes was measured using an EG&G nitrogen laser equipped with a dye-module [model 2100] containing Rhodamine 6G as an excitation source. This laser system

produces pulses at 595 nm with a pulse width of approximately 1 ns at a repetition rate of 1–30 Hz. Fluorescence transients were detected at 90° with an avalanche photodiode (active area 1 mm^2, Hamamatsu Ltd.). The signal was digitized with a 5 Gs/s, 600 MHz digital oscilloscope (LeCroy model 9360), and 256 traces were averaged per sample to obtain an acceptable signal to noise ratio. In a typical experiment the instrument response curve was measured first, detecting the scattered light through an interference filter at 460 nm. The fluorescence signal was measured through an interference filter at 910 nm before and after the addition of 10–50 μM sodium ascorbate or sodium dithionite to pre-reduce Q_A. The samples were subsequently reoxidized with 1 mM potassium ferricyanide and a final trace was measured to ensure that the reduction of Q_A was reversible. The fluorescence decay curves were analyzed by linear least squares fits of the actual measurement to a convolution of the instrument function and a fast and slow decay component. The two decay components were varied linearly in 50 or 100 discrete steps over a relevant interval of both t_1 and t_2. With these parameters fixed the optimal A1 and A2 were determined at each combination of t_1 and t_2 and the optimal set of parameters was determined from a two-dimensional $\chi 2$ plot. Generally only one well defined minimum was observed for time ranges of t_1 between 0.1 and 1 ns and of t_2 between 1 and 20 ns. Since it was not experimentally possible to accurately determine the absolute fluorescence intensities for each of the samples, the fluorescence signals were normalized and the relative amplitudes of the fast and slow decay times were determined. For the WT and all of the mutants the fast decay component was less than 1 ns and was considered to be limited by the instrument response function.

The results of delayed fluorescence measurements were analyzed using the 4-state kinetic model for the electron transfer outlined in Figure 1. In this model the mixed antenna/RC excited state $(AP)^*$ decays through two channels, fluorescence and trapping (resulting in subsequent charge separation to $P^+H_L{}^-$). In view of the time-resolution of the experiment no discrimination is made between excited state of the antenna (A*P) or of the special pair (AP*). Consequently, the forward reaction (k) in this model reflects the rate of trapping rather than the rate of primary electron transfer. The recombination rate (k_{-1}) is calculated as a function of the free energy difference ΔG° between the states $(AP)^*$ and $P^+H_L{}^-$ using the Boltzmann equation. The model further incorporates loss of the $P^+H_L{}^-$ state by the formation of triplet states (q). For this model a set of 4 differential equations were solved using the computer program Mathematica, which allows the direct derivation of values for ΔG° from the experimental results (see legend to Figure 1) using literature values of $8.0 \cdot 10^8$ $s^{-1}$for the rate of decay of excited state fluorescence (Sebban et al. 1984); and $1 \cdot 10^8$ s^{-1} for the rate of triplet formation (Schenck et al. 1982).

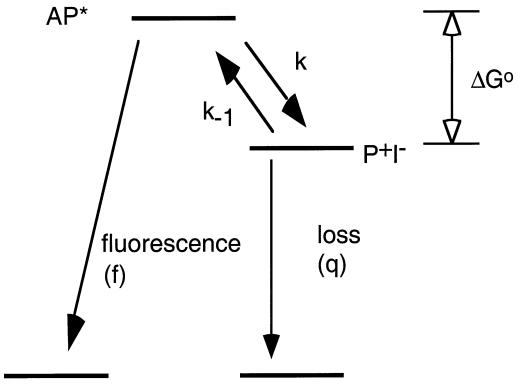

Figure 1. Schematic representation of a 4-state model used to describe primary electron transfer in the RC. The excited state $(AP)^*$ decays through two channels, fluorescence (f) and primary electron transfer (k). The rate of charge recombination k_{-1} is calculated as a function of ΔG° using the Boltzmann equation. The charge separated state is allowed to decay by an intrinsic rate q representing the sum of triplet formation and radiation-less decay. For the antenna fluorescence f a rate constant of 1.25×10^9 s^{-1} was taken (Sebban et al. 1984); q was set to 1×10^8 s^{-1} (Scheck et al. 1982) and assumed to be the same for all mutants. The following set of differential equations was solved using Mathematica: Q′[t] − qX[t] == 0; X'[t] + qX[t] + mX[t] −kA[t] == 0, F′[t] − zA[t] == 0; A′[t] + zA[t] + kA[t] −mX[t] == 0; using A[t] + X[t] + Q[t] + F[t] == 1 as a boundary condition and the following starting values: A[0] == 1; X[0] == 0; Q[0] == 0; F[0] == 0.

Results

Mid-point redox potentials of P in RC-only and RCLH1 strains

Table 1 summarises the mid-point potentials for the P/P^+ redox couple (E_m P/P^+) that were obtained for the RCLH1 and RC-only membranes containing WT and mutant RCs. In all cases the titrations could be fitted satisfactorily with an n = 1 Nernst curve. As discussed in detail in (Beekman et al. 1996), the values that were obtained for the RC-only versions of the WT, YM210F, YM210H and YM210W mutants are in good agreement with values reported by other groups for purified WT and mutant RCs (Moss et al.

1991; Williams et al. 1992; Jia et al. 1993; Murchison et al. 1993; Nagarajan et al. 1993; Lin et al. 1994; Peloquin et al. 1994), demonstrating that the presence of the membrane in the RC-only samples had no significant effect on E_m P/P^+. In contrast, the LH1 antenna complex has a small but reproducible effect on E_m P/P^+. Although the RCLH1 membrane samples showed the same trend of a higher redox potential than WT in the YM210F, YM210L and YM210W mutants, and a lowered potential in the YM210H and YM210H/FL181H mutants, in all cases the primary donor in RCLH1 membranes had a significantly lower E_m P/P^+ than its RC-only counterpart, with most of the mutants showing about a 30 mV difference (Table 1). The variation in the size of the 'LH1 effect' on the redox potential was not attributable to significant variations in the ratio of LH1:RC in the samples of RCLH1 membranes, judged from the absorption spectrum (McGlynn et al. 1994), since the amount of LH1 per RC was very similar in all of the RCLH1 strains examined (Beekman et al. 1994).

One possible source of the observed LH1 effect could be a change in the ability of the redox-mediators used in the titration to poise the RC in the two types of membrane sample, for example as a result of differences in membrane morphology RCLH1 membranes are tubular, although they may not be sealed, whereas RC-only membranes are open sheets (Jones et al. 1992). However, we did not observe any dependence of E_m P/P^+ on the concentration of any of the mediators, and all titrations were fully reversible, indicating proper redox equilibrium in the sample. Also, it seems unlikely that the lateral presence of the antenna complexes will strongly influence the accessibility of P for small mediator molecules in the aqueous phase, as P lies close to the periplasmic face of the RC protein, that is exposed to the solvent in both types of membranes. Therefore, we conclude that this difference in measured redox potentials is an intrinsic effect of the presence of the LH1 complex.

Kinetic analysis of delayed fluorescence from Q_A-reduced RCs

Fluorescence emission on a nanosecond timescale was measured in RCLH1 membranes in which the Q_A quinone had been reduced, as described in Materials and Methods. A typical kinetic trace showing the decay in fluorescence emission is shown in Figure 2. All traces were fitted with a convolution of the shape of the excitation pulse and a bi-exponential decay, as

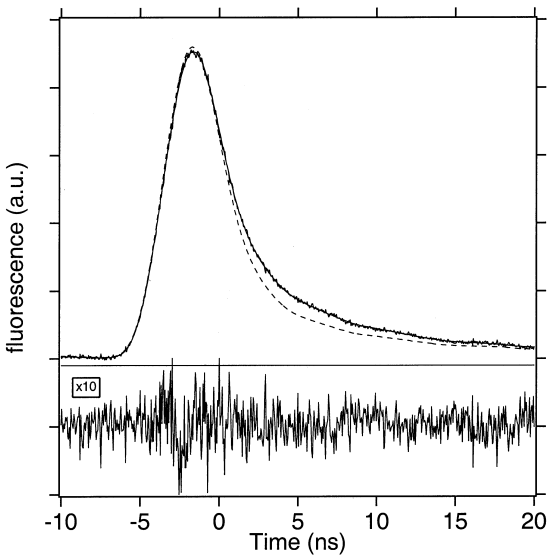

Figure 2. Fluorescence decay curves for the YM210F(RCLH1) mutant measured with intracytoplasmic membranes under oxidizing (dashed) and reducing (solid) conditions (top panel). The instrument response function as measured through an interference filter at 460 nm was in all cases close to identical to the signal measured under oxidizing conditions, except for an offset in time caused by the different response of the avalanche photodiode to different colors of light. The bi-exponential fit to the measurement under reducing conditions is difficult to observe, and therefore the residuals have been expanded 10 times and offset (bottom panel).

described in 'Materials and methods'. Details of the kinetic fits are given in Table 1; in all cases a bi-exponential decay provided an adequate fit to the data. In those RCs where E_m P/P^+ was raised relative to the WT RC, and where it is known that the rate of primary charge separation is slowed (see Table 1), there was a relative increase in the amplitude of the slower of the two fluorescence components.

The fluorescence emission data were used to obtain an estimate of ΔG° for charge separation in the WT and in those mutants that showed an increase in the relative amplitude of the slow phase of emission. Fluorescence emission from RCs in which the Q_A quinone has been reduced consists of a fast (picosecond) phase that decays with the kinetics of charge separation and which originates from the excited state population that is formed initially, and one or more slow (nanosecond) phases that arise from thermal repopulation of the excited state from the charge-separated state $P^+H_L^-$ (Woodbury and Parson 1984, 1986; van Grondelle et al. 1987). The relative amount of delayed fluorescence observed is therefore sensitive to the magnitude of the free energy gap between the excited state and charge-

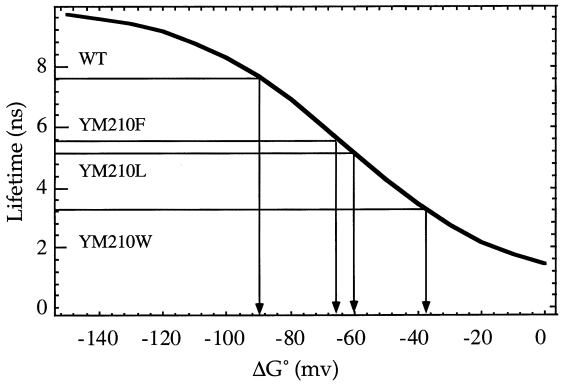

Figure 3. Relation between the observed slow component of the fluorescence lifetime t_2 and free energy difference between (AP)* and $P^+H_L^-$ obtained by solving the differential equations outlined in the legend to Figure 1. The parameters used where $k_1 = 2.2 \times 10^{10}\ s^{-1}$; $f = 1.25 \times 10^9 s^{-1}$. The backrate k_{-1} was calculated from the free energy difference. The ΔG° values obtained for the different mutants from their slow fluorescence lifetime component are indicated.

separated state, and so measurement of the relative amplitude of prompt and delayed emission can give an estimate of the free energy difference between, in these experiments with RCLH1 complexes, (AP)* and $P^+H_L^-$.

The ΔG° for the primary step was estimated from the fluorescence decay curves according to three different procedures, the results of which are summarized in Table 2. In the first method, the ΔG° for charge separation was directly estimated from the ratio of the initial amplitudes of the fast and slow components of the fluorescence decay, since this ratio reflects the equilibrium constant under the assumption that the (AP)* and $P^+H_L^-$ states are fully equilibrated on the measured timescale (Schenck et al. 1982; Woodbury and Parson 1984). Since resolution of the fast component is limited by the time response of the instrument, this method is expected to underestimate the amplitude of the fast component and therefore underestimates the value for ΔG° in the WT and mutant membranes (shown in column A of Table 2). These values are presented to illustrate the size of the correction that results from the second method. In the second method, this lack of time resolution was corrected for by calculating the integrated fluorescence from the fast component and dividing that by the expected lifetime of the stimulated emission that gives rise to the fast signal, which in this case is the trapping time of the individual mutants as determined in Beekman et al. (1994). The results from this method are shown in column B of Table 2. In the third method, values for ΔG° were estimated from the 4-state kinetic model described in Materials and methods and Figure 1. The differential equations that describe this 4-state model were solved, and the expected recombination rates and proportions of prompt and delayed fluorescence were calculated as a function of the ΔG° between AP* and $P^+H_L^-$. The relation between ΔG° and the lifetime of the delayed fluorescence obtained by this approach is shown in Figure 3. This relation was used to estimate ΔG° from the observed rate of decay of the recombination fluorescence (shown in column C of Table 2). Although the absolute values for ΔG° obtained by the three methods showed some variation, all the methods yielded similar changes in driving force ($\Delta\Delta G^\circ$) for the mutants with respect to the WT. These in turn were in good agreement with the changes in driving force that would be expected to arise from the change in redox-potential of P/P^+ measured for these mutants (shown in column D of Table 2).

Discussion

Since the determination of the atomic structure of the bacterial RC, considerable research effort has focussed on the parameters that govern the rate of light-driven electron transfer in the complex, such as the magnitude of the driving force for electron transfer (ΔG°) and the reorganization energy (λ) of the surrounding medium. This research, often employing mutated RCs, has centered upon electron transfer in 'antenna-free' RCs, for the practical reasons outlined in the Introduction. However, as the bacterial RC has evolved to operate in the presence of the LH1 antenna complex, it is of interest to examine whether the LH1 complex influences the properties of the RC, and in particular whether the parameters that govern the rate of transmembrane electron transfer are sensitive to the presence of the LH1 antenna.

The rate of electron transfer is affected by the redox potential of the RC primary donor, which in part determines the free energy of the charge-separated states $P^+H_L^-$ and $P^+Q_A^-$. A number of groups have measured the value of E_m P/P^+ in the WT *Rb. sphaeroides* RC, using both chemical and electrochemical titrations. Consideration of the literature on this topic reveals an interesting observation. Titrations carried out on chromatophore membranes from WT (antenna-containing strains) tend to show a value for E_m P/P^+ of approximately +445 mV (Dutton and Jack-

Table 2. Energy-gaps (ΔG°) and relative changes ($\Delta\Delta G^\circ$) from wild type YM210

RCLH1 mutant	A ΔG° (meV)	A $\Delta\Delta G^\circ$ (meV)	B ΔG° (meV)	B $\Delta\Delta G^\circ$ (meV)	C ΔG° (meV)	C $\Delta\Delta G^\circ$ (meV)	D ΔE_m P/P^+ (meV)
YM210 (wt)	–110		–169		–90		
YM210F	–83	27	–140	29	–60	30	20
YM210L	–88	22	–135	34	–66	24	26
yM210W	–62	48	–108	60	–38	52	45

A: values determined from the ratio of amplitudes of fast and slow component.
B: ratio of the corrected fast and slow component (see text).
C: values derived from the kinetic model.
D: change in mid-point redox potential relative to the RCLH1 WT control.

son 1972; Jackson et al. 1973), whilst titrations carried out on purified RCs show a range of values between +485 mV and +510 mV (Moss et al. 1991; Williams et al. 1992; Murchison et al. 1993; Peloquin et al. 1994). This discrepancy of approximately 50 mV is not attributable to detergent effects or removal of the RC from the membrane, as titrations performed with RC-only membranes also reveal a redox potential of close to +490 mV for the WT RC (Beekman et al. 1995).

The titrations described in this report show that the LH1 antenna complex has a small but significant influence on E_m P/P^+. This effect is seen not only in the WT RC, but also in a set of RC mutants in which E_m P/P^+ is altered over a 130 mV range, with a reduction of about 30 mV being seen in most of the RCs studied. We are currently examining whether the peripheral LH2 antenna complex has an additional effect on E_m P/P^+. In the absence of detailed structural information on the RC/LH1 core complex it is difficult to ascribe the effect of LH1 on the properties of P to a specific molecular interaction or change in RC conformation, other than to say that it seems likely that the presence of the core antenna affects the local environment of P. It is interesting to note that parts of the macrocycle of both P and H_L are relatively close to the edge of the RC structure and might therefore directly interact with the LH1 antenna (Deisenhofer et al. 1985; Allen et al. 1987; Ermler et al. 1994).

The estimates of ΔG° obtained from the fluorescence recombination kinetics for the RCLH1 versions of the 'slowed' M210 mutants show considerable variation (columns A to C in Table 2), reflecting the assumptions that were made in their calculation. In model A, ΔG° is underestimated since the fast component is not fully resolved. Model B corrects for this by using the integrated fluorescence and the known trapping rates for these mutants (from Beekman et al. 1994). Although heterogeneous trapping was reported for mutants from *Rb. capsulatus* (Laible et al. 1997) it appears that describing the prompt emission from our mutants as single exponential is accurate enough for the present purpose. Both model B and C use a single exponential term to describe the delayed component, even though multiphasic decays have been reported and analysed in detail. These multiphasic kinetics have been ascribed to time-dependent relaxation of the $P^+H_L^-$ state and and to triplet formation (Woodbury et al. 1984, 1986, 1994; Goldstein and Boxer 1989a, b) and to heterogeneity in the primary electron transfer resulting from heterogeneity in the free energy of $P^+B_L^-$ (Hartwich et al. 1998). Our data on recombination fluorescence was satisfactorily described by a single exponential decay, and therefore a model in which only one final state of $P^+H_L^-$ gives rise to the delayed emission was sufficient to explain our data. Finally, it should be noted that in our measurements the delayed component is weighted in favor of the subpopulations of $P^+H_L^-$ with the highest energy (Ogrodnik et al. 1994). Therefore, it is possible that a significant change in the heterogeneity of this level would give rise to similar effects without changing the mean free energy of the total population.

In the light of these considerations, the absolute values for ΔG° presented in Table 2 should be regarded with caution. However, the differences calculated between the WT and the YM210F, YM210L and YM210W mutants ($\Delta\Delta G^\circ$) are in good agreement with the changes in E_m P/P^+ measured for these mutants (compared in column D of Table 2), regardless of how the absolute values for ΔG° were calculated. The most obvious conclusion from this is that the change in ΔG° measured for these mutants relative to the WT stems largely from the measured change in E_m P/P^+ and that any effects of the muta-

tions on the reduction potential of the H_L/H_L^- redox couple (which also contributes to the free energy of the $P^+H_L^-$ state), do not contribute significantly to the change in $\Delta G°$. This conclusion is broadly in line with the work of Nagarajan et al. (1993) who concluded that changes in the driving force for the reaction $P^* \rightarrow P^+H_L^-$ in purified YM210F, YM210I and YM210W RCs stemmed largely from a measured change in E_m P/P^+. However, it should be noted that changes in the rate of the reaction $P^+H_L^- \rightarrow P^+Q_A^-$ were reported by Nagarajan et al (1993) for purified YM210F, YM210I and YM210W RCs and by Beekman et al (1996) for membrane-bound YM210L and YM210W RCs, and were attributed to an effect of the mutations at the YM210 position on the potential of the H_L/H_L^- redox couple. One possible explanation for these contradictary observations is that this interpretation is incorrect, and the rate of secondary electron transfer in these RCs is in fact affected by a change in another parameter, such as λ for the reaction. Alternately, it may be that the YM210L and YM210W mutations affect the redox properties of H_L in RCs in RC-only membranes or purified complexes, but not in RCs in RCLH1 membranes. Finally, this discrepancy may arise from differences in the experimental conditions employed, the rate of secondary electron transfer being derived from absorbance difference spectroscopy on the picosecond time scale with Q_A oxidised (Nagarajan et al. 1993; Beekman et al. 1996), whilst estimates of $\Delta G°$ were made from fluorescence measurements on the nanosecond timescale with Q_A reduced. If, as proposed, there is a relaxation of the free energy of the $P^+H_L^-$ state (Woodbury et al. 1994), it is also possible that the mutations at the M210 position have different effects on E_m of H_L/H_L^- in the $P^+H_L^-$ state formed initially (and that leads to $P^+Q_A^-$) than in the relaxed $P^+H_L^-$ state formed when Q_A is reduced (Nagarajan et al. 1993). This is clearly a point that warrants further investigation.

We now turn to the question of whether the absolute value of $\Delta G°$ for charge separation is modulated by the presence of the antenna. Applying the most simple of interpretations, our findings on E_m P/P^+ would suggest that $\Delta G°$ is approximately 30 mV larger in the presence of the LH1 antenna than in its absence. As far as we are aware, there is no unequivocal evidence that the LH1 antenna has a direct influence on the redox properties of H_L, although we cannot exclude this possibility. The RC bacteriopheophytines are located close to the intra-membrane surface of the RC, and may come into close contact with the LH1 antenna. The other parameter that determines the $\Delta G°$ that drives charge separation is the energy of the excited state P*. In an earlier paper (Beekman et al. 1994) we have argued that in order to achieve efficient energy transfer, the excited state of the core-antenna bacteriochlorophylls (A^*) and that of the RC primary donor (P^*) are nearly iso-energetic, and this has the effect of lowering the free energy of the excited state $(AP)^*$. As discussed previously (Beekman et al. 1994), if it is assumed that there are approximately 12 antenna dimers surrounding P, in broad agreement with measurements on membranes of this sort (Francke and Amesz 1995; McGlynn et al. 1996), this decrease in the free energy of the $(AP)^*$ state will amount to some 66 meV, and will therefore be a substantial effect. This lowering of the free energy of the excited state due to the presence of LH1 would lead to a decrease in the $\Delta G°$ for charge separation, in contrast to the increase in $\Delta G°$ brought about through the influence of LH1 on the redox properties of P. The net result of these counteracting effects of the LH1 antenna would therefore be a decrease of approximately 35 meV in the $\Delta G°$ for charge separation. In antenna-free RCs, increases in $\Delta G°$ of the order of a few tens of meV (implied by a decrease of a few tens of mV in the measured E_m P/P^+) have the effect of slightly accelerating the rate of P* primary electron transfer (Chan et al. 1991; Jia et al. 1993; Beekman et al. 1996).

To summarise, it seems likely that the LH1 antenna (and possibly also LH2) influences the energetics of charge separation in RCs through a combination of small, but readily observable, effects on the excited state energy and reduction properties of the cofactors involved, which alter the $\Delta G°$ for charge separation. These modulations are of a magnitude that would be sufficient to explain why the rate of primary charge separation is not optimal in antenna-free WT RCs, and can be accelerated by mutations that bring about a small increase in the driving force in the reaction, although we note that there is no a priori reason to believe that the rate of primary electron transfer should be optimal in the WT RC. The results demonstrate that *in vivo* the RC and its antenna comprise an intimate functional unit in which the pigments and/or protein subunits of LH1 modulate the properties of the RC.

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