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Free-energy change accompanying the reduction of the reaction center secondary quinone in *Rhodopseudomonas sphaeroides* chromatophores

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The standard free-energy change accompanying the electron transfer from Q_A to Q_B was estimated from the intensity of the delayed fluorescence in chromatophores of *Rhodopseudomonas sphaeroides*. The value of 120 meV (at pH 8) suggests that Q_B^- is more stable in the chromatophore membrane than in the isolated reaction center.

The primary charge separation in bacterial photosynthesis takes place in a pigment-protein complex called reaction center. The electron donor is a bacteriochlorophyll dimer (P), and the first stable acceptor is a quinone molecule [1,2]. The reaction center of *Rhodopseudomonas sphaeroides* binds two ubiquinone molecules. The primary acceptor quinone, Q_A, accepts one electron to form a semiquinone anion radical and does not accept the second electron under normal conditions. The secondary acceptor quinone, Q_B, is reduced by electrons from Q_A, and is stable in both semiquinone and quinol forms. Thus, two electrons from Q_A are combined on Q_B and transferred out of the reaction center [3,4]. This function of Q_B as a two-electron gate is established in the isolated reaction center. In the intact chromatophore membrane, on the other hand, the expected binary patterns in the semiquinone formation, the reduction of cytochrome *b* or the proton uptake have been observed only under certain conditions [5–8]. Bowyer and Crofts [9] suggested that in half of the reaction centers Q_B was reduced to semiquinone with an apparent midpoint potential of 350 mV (pH 7). However, the mechanism that stabilizes Q_B^- is not yet understood.

For the standard free-energy change, ΔG_A^B, accompanying the electron transfer

$$Q_A^- Q_B \rightleftharpoons Q_A Q_B^- , \qquad (1)$$

values of 70–80 mV were obtained with several independent techniques, using isolated reaction centers from *Rps. sphaeroides* R-26 [10–14].

In the present paper, I applied the method of delayed fluorescence [11] to estimate the value of free-energy change in the intact chromatophore. The results suggests that Q_B^- is thermodynamically more stable in the chromatophore membrane than in the isolated reaction centers.

Cells of *Rps. sphaeroides* strain 2.4.1 were cultured in the medium described by Cohen-Bazire et al. [15]. Chromatophores free of cytochrome c_2 were prepared as described previously [16]. Delayed fluorescence was measured with the phosphoroscope described in [16]. Samples were excited by a 10 μs xenon flash (MF-1500-U3, Sugawara Laboratories) in combination with optical filters, Corning 9788 and Toshiba VO-55. The temperature of the sample was kept at 36°C in the measurement of the delayed fluorescence, because it depended strongly on the temperature [11]. No

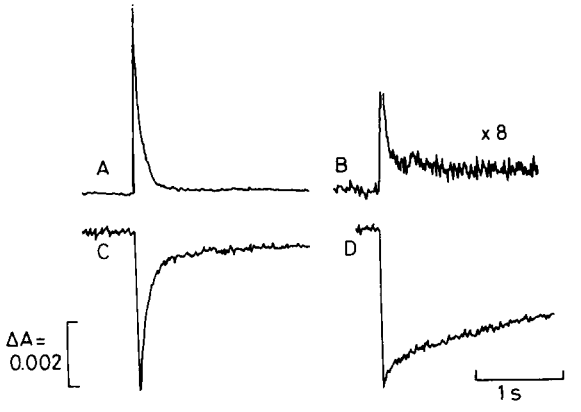

Fig. 1. Time-courses of delayed fluorescence and absorbance change at 605 nm after the flash excitation. Traces A and B, delayed fluorescence. The shutter in front of the photomultiplier was opened 10 ms after the flash excitation. The vertical scale of trace B is expanded eight times relative to that of trace A. Traces C and D, absorbance change at 605 nm. The downward deflection corresponds to the decrease of the absorbance. The signals were averaged eight times for traces A, C and D, and 32 times for trace C. Cytochrome c_2-free chromatophores (15 μM bacteriochlorophyll) were suspended in 10 mM Tris-HCl (pH 8.0)/100 mM KCl. 2 μg/ml gramicidin D was added. (B) and (D), no further addition; (A) and (D), 2 mM *o*-phenanthroline was added.

delayed fluorescence was detected at room temperature.

Fig. 1 shows typical time-courses of the delayed fluorescence, together with those of the rereduction of P^+. In the presence of 2 mM *o*-phenanthroline, the electron transfer from Q_A to Q_B was inhibited and P^+ was rereduced by Q_A^- with a rate constant (k_{AB}) of about 15 s^{-1} (trace C). About 20% of the reaction center was not inhibited, and decayed slowly, probably because the affinity of *o*-phenanthroline to the reaction center is not high enough [10,17]. The fluorescence had a decay kinetics similar to the fast component of the P^+ reduction (trace A), as described previously [16]. In the absence of the inhibitor, P^+ decayed slowly with an apparent rate constant (k_{app}) of about 0.2 s^{-1} (trace D). The decay did not simply follow the first-order kinetics, however. Under the same conditions, the fluorescence was very weak and decayed somewhat faster (0.5–1 s^{-1}) than P^+. The analysis of the decay kinetics was, however, difficult because of a low signal-to-noise ratio. The small fast-decaying component in trace B is an artifact: it did not depend on the temperature of the sample and was observed without chromatophores. The artifact decayed within 100 ms and did not disturb the measurements of the slow-decaying fluorescence.

For the estimation of ΔG_A^B, it was assumed that the intensity of the fluorescence, F, was determined by the amount of state $P^+Q_A^-Q_B$:

$$F = k_F K_A^* [P^+ Q_A^- Q_B], \tag{2}$$

where k_F is the rate constant for the radiative loss of the excitation energy of P, and K_A^* is the equilibrium constant for the excitation of P by the charge recombination of the radical pair $P^+Q_A^-Q_B$ [11]. The equilibrium of Eqn. 1 is established rapidly (100 μs) after the excitation, and is maintained during the relaxation [11]:

$$\frac{[P^+ Q_A Q_B^-]}{[P^+ Q_A^- Q_B]} = K_A^B, \tag{3}$$

where $K_A^B (= \exp(-\Delta G_A^B/RT))$ is the equilibrium constant of the reaction. Therefore,

$$F = k_F K_A^* \frac{[P^+ Q_A^- Q_B] + [P^+ Q_A Q_B^-]}{1 + K_A^B} = k_F K_A^* \frac{[P^+]}{K_A^B}. \tag{4}$$

In the reaction centers inhibited by *o*-phenanthroline, electrons stay on Q_A, and thus:

$$F = k_F K_A^* [P^+]. \tag{5}$$

The ratio of the value $F/[P^+]$ in the absence of the inhibitor (Eqn. 4) to that in the presence (Eqn. 5) gives the equilibrium constant K_B^A. The intensity of the fluorescence was extrapolated to the time of the flash excitation on semi-logarithmic plots of the time-courses (Fig. 2). The fast component in the decay of P^+ was used for $[P^+]$ of the inhibited reaction centers. From the obtained value of K_A^B, ΔG_A^B was calculated to be -120 meV. The free-energy change was considerably larger than the value obtained with the isolated reaction centers [10–14].

Since the standard free energy of the radical pair $P^+Q_A^-$ in the chromatophore (0.51 eV above the state PQ_A [16]) is not significantly different from that in the isolated reaction center (0.52 eV [11]), the state $P^+Q_AQ_B^-$ appears to be more stable in the chromatophore membrane than in the isolated reaction center.

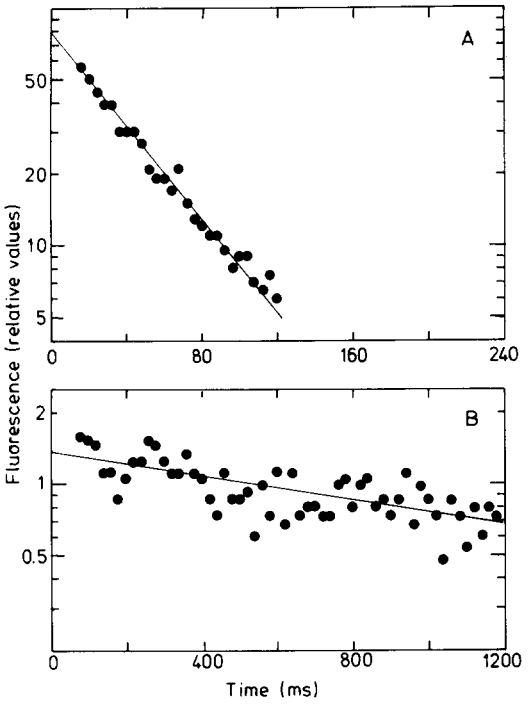

Fig. 2. Semi-logarithmic plot of the time-courses of delayed fluorescence. Experimental conditions were as in Fig. 1. 2 mM *o*-phenanthroline was added in A.

One possible interpretation for the low free-energy level is that the electrons are distributed out of the reaction center within a few milliseconds. If half of the reaction centers had Q_B in the semiquinone form before the flash, as suggested by Bowyer and Crofts [9], only the remaining half would contribute to the observed delayed fluorescence. Then, the standard free-energy change would be -100 meV, which is still quite different from the value in the isolated reaction centers. However, it is not clear if the electrons were transferred out of the reaction centers in the chromatophores free of cytochrome c_2. Under the present conditions for delayed fluorescence measurements, no reduction of cytochrome *b* was observed (data not shown).

Since the standard free energy of the state $P^+Q_A^-$ is 0.51 eV relative to the ground state PQ_AQ_B [16], the standard free energy of the state $P^+Q_AQ_B^-$ is 0.39 eV above the ground state. The midpoint redox potential of P is about 450 mV. Therefore, the midpoint potential of Q_B is about 60 mV, if the interaction between P^+ and Q_B^- is negligible. The value agrees within the experimental error with the midpoint potential obtained by the chemical titration (40 mV at pH 8 [18]).

Another supporting evidence for the idea that Q_B^- is thermodynamically more stable in chromatophores has been mentioned by Wraight [19]. In the redox titration of Q_B^- EPR signal in chromatophores, semiquinone appeared with midpoint potential of $+40$ mV and disappeared with that of -15 mV (pH 8.0) [18], while Q_B in isolated reaction centers showed two-electron character ($E_{m,8} = -5$ V) [19].

Although the functional midpoint potential for the Q_B^-/Q_B couple appeared to be very high, it is not as high as the one reported for the disappearance of the binary patterns [7,8]. Probably, Q_B^- is stable kinetically, too, and an electron is trappped on Q_B^- when an effective electron donor to P exists.

Fig. 3 shows ΔG_A^B as a function of pH. It decreased with increasing pH with an apparent pK of 6.8. This suggests that Q_B^- associates with a proton with a pK value of 6.8 and Q_A^- does not. Since the standard free energy of the state $P^+Q_A^-$ did not depend on pH [16], the photoreduction of Q_A may not accompany protonation. Chemically

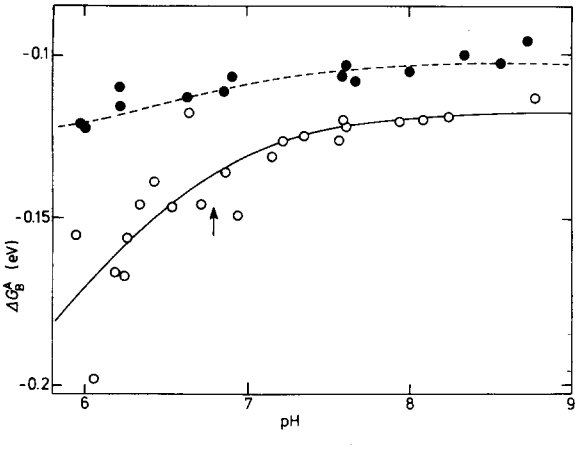

Fig. 3. The standard free-energy change of the electron transfer from Q_A to Q_B, as a function of pH. Chromatophores were suspended in 10 mM phosphate buffer with 100 mM KCl. 2 μg/ml gramicidin D was added. The standard free-energy change was calculated from the intensity of the delayed fluorescence (○) or the decay rate constant of P^+ (●). The solid line was drawn assuming that Q_B^- binds a proton with a pK of 6.8 (indicated by an arrow).

titrated E_m of Q_A depended on pH [17,18,20], but this could be the results of protonation following slow relaxation to the redox equilibrium [20,21].

Kleinfeld et al [14] estimated ΔG_A^B as a function of pH with isolated reaction centers and argued that Q_A^- and Q_B^- associate a proton with pK values of 9.8 and 11.3, respectively. The relation of their results with mine is so far not clear. If the proton binds to a basic amino acid residue interacting with the quinone molecules, as suggested by Wraight [22], the interaction could be different in the chromatophore membranes. Another possibility is that Q_B^- transfers the electron to the quinone pool or the cytochrome b-c_1 complex at lower pH's than 6.8.

The observed pK value for Q_B^- was lower than the values obtained by Petty and Dutton [23] from direct proton uptake measurements. In conditions similar to my delayed fluorescence measurements (at redox potential of 380 mV), apparent pK of Q_B^- was 7.5. Cogdell et al. [24] also measured the uptake of protons with aerobic suspension of chromatophores and observed only slight decrease in the amount of protons taken up with increasing pH up to pH 8. The origin of the descrepancy is, however, not clear. I measured the uptake of protons under the same conditions as the delayed fluorescence measurements with the same preparation, using Phenol red and bromcresol purple as pH indicators. The results were rather similar to those by Petty and Dutton [23] (data not shown).

The closed circles in Fig. 3 show apparent values of ΔG_A^B estimated using k_{AB} and k_{app} [10,14]. Since the decay did not simply follow first-order kinetics in the absence of o-phenanthroline, I used the pseudo-first-order rate constant, $(d[P^+]/dt)/[P^+]$, at early stage (0–1 s) of the decay for the value of k_{app}. The difference with the values from the delayed fluorescence was probably due to the direct reduction of P^+ by electrons from Q_B or by other electron donors [25]. These processes could also cause the complicated decay kinetics of P^+. In the isolated reaction centers, on the other hand, the recombination of electrons from Q_B^- is much faster, and the competing processes may not contribute significantly to the rereduction of P^+.

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References

1 Feher, G. and Okamura, M.Y. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 349–386, Plenum Press, New York
2 Parson, W.W. and Ke, B. (1982) in Photosynthesis (Govindjee, ed.), Vol. I, pp. 331–385, Academic Press, New York.
3 Vermeglio, A. (1977) Biochim. Biophys. Acta 459, 516–524
4 Wraight, C.A. (1977) Biochim. Biophys. Acta 459, 525–531
5 Barouch, Y. and Clayton, R.K. (1977) Biochim. Biophys. Acta 462, 785–788
6 De Grooth, B.G., Van Grondelle, R., Romijn, J.C. and Pulles, M.P.J. (1978) 503, 480–490
7 Bowyer, J.R., Tierney, G.V. and Crofts, A.R. (1979) FEBS Lett. 101, 201–206
8 O'Keefe, D.P., Prince, R.C. and Dutton, P.L. (1981) Biochim. Biophys. Acta 637, 512–522
9 Bowyer, J.R. and Crofts, A.R. (1981) Biochim. Biophys. Acta 636, 218–233
10 Wraight, C.A. and Stein, R.R. (1980) FEBS Lett. 113, 73–77
11 Arata, H. and Parson, W.W. (1981) Biochim. Biophys. Acta 638, 202–209
12 Mancino, L.J., Dean, D.P. and Blankenship, R.E. (1984) Biochim. Biophys. Acta 764, 46–54
13 Kleinfeld, D., Abresch, E.C., Okamura, M.Y. and Feher, G. (1984) Biochim. Biophys. Acta 765, 406–409
14 Kleinfeld, D., Okamura, M.Y. and Feher, G. (1984) Biochim. Biophys. Acta 766, 126–140
15 Cohen-Bazire, G., Sistrom, W.R. and Stanier, R.Y. (1975) J. Cell Comp. Physiol. 49, 25–68
16 Arata, H. and Nishimura, M. (1983) Biochim. Biophys. Acta 725, 394–401
17 Wraight, C.A. (1981) Isr. J. Chem. 21, 348–354
18 Rutherford, A.W. and Evans, M.C.W. (1980) FEBS Lett. 110, 257–261
19 Wraight, C.A. (1979) Photochem. Photobiol. 30, 767–776
20 Prince, R.C. and Dutton, P.L. (1976) Arch. Biochem. Biophys. 172, 329–334
21 Blankenship, R.E. and Parson, W.W. (1979) in photosynthesis in Relation to Model Systems (Barber, J., ed.), Vol. 3, pp. 71–114, Elsevier, Amsterdam.
22 Wraight, C.A. (1979) Biochim. Biophys. Acta 548, 309–334
23 Petty, K.M. and Dutton, P.L. (1976) Arch. Biochem. Biophys. 172, 335–345
24 Cogdell, R.J., Jackson, J.B. and Crofts, A.R. (1973) Bioenergetics 4, 211–227
25 Blankenship, R.E. and Parson, W.W. (1979) Biochim. Biophys. Acta 545, 429–444