Volume 233, number 2, 331–334 FEB 05954 June 1988

Activation energy of the rate constant of $P^+Q_A^-$ absorption decay in reaction centers from *Rhodobacter sphaeroides* reconstituted with different anthraquinones

Pierre Sebban

Laboratoire de Photosynthèse du CNRS, ER 307, Bat. 24, CNRS, Gif-sur-Yvette 91190, France

Received 21 April 1988

The free energy differences between the first stable charge-separated state, $P^+Q_A^-$ and the thermally excited state, M, via which $P^+Q_A^-$ recombines, were measured in the reaction centers from *Rhodobacter sphaeroides* reconstituted with anthraquinones. We find that the free energy level of M is independent of the in situ redox potential of Q_A and consequently the free energy level of $P^+Q_A^-$. This suggests that M is a state independent of $P^+Q_A^-$. This is in support of the identification of M with a relaxed form of P^+I^-.

Bacterial photosynthesis; Reaction center; Recombination kinetics; Energetics; Quinone

1. INTRODUCTION

The light excitation energy absorbed by the antennae of photosynthetic organisms is converted into chemical free energy at the level of the reaction centers. The first step of this process is the excitation of a dimer of bacteriochlorophyll (P) to its first singlet excited state, P*. Then, an electron is transferred in about 3 ps [1,2] to a molecule of bacteriopheophytin (I). The first charge separation state, P^+I^-, decays within about 230 ps [3–5], the time needed for the electron to reduce the secondary electron acceptor, a quinone molecule (Q_A). Under normal conditions, the electron is transferred to a secondary quinone, Q_B. When Q_B is extracted or in the presence of an inhibitor of the Q_A-to-Q_B electron transfer, charge recombination between P^+ and Q_A^- is induced. In native reaction centers from the purple bacterium *Rhodopseudomonas viridis,* this recombination occurs in about 1 ms at pH 9 [6–8]. In the reaction centers from *Rhodobacter sphaeroides,* this process is much slower ($\approx$100 ms). This was interpreted in terms of different ways for P^+ and Q_A^- to recombine in these two strains [6]. It was proposed [6–14] that, in the reaction centers, the charge recombination between P^+ and Q_A^- occurs either directly to the ground state *(R. sphaeroides)* or via a thermally excited state, M. *(Rps. viridis),* depending on the free energy difference (ΔG^*) between the states $P^+Q_A^-$ and P* [14]. In the reaction centers from *R. sphaeroides,* where the native Q_A (ubiquinone-10) was replaced by various quinones of very different midpoint redox potential, Woodbury et al. [14] estimated that if $\Delta G^* < 0.8$ eV, $P^+Q_A^-$ mainly recombines via M, whereas if $\Delta G^* > 0.8$ eV, back electron transfer occurs directly. In this study, the differences in the observed ΔG^* values corresponded to the differences in the in situ redox potential of the various quinones.

In *Rps. viridis* reaction centers, summation of the free energy differences between pQ_A and $P^+Q_A^-$, 0.65 eV [15], with that between $P^+Q_A^-$ and M, 0.29 eV [6,8], and between P^+I^- and P*, 0.25 eV [16], gives a value close to 1.24 eV for the energy of the $pQ_A \longrightarrow p^*$ transition. This is in sup-

Correspondence address: P. Sebban, Laboratoire de Photosynthèse du CNRS, ER 307, Bat.24, CNRS, Gif-sur-Yvette 91190, France

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

port of the identification of M with P^+I^-, assuming some relaxation of P^+I^- in the micro- and millisecond time ranges [14]. This hypothesis is not supported by the data available on *R. sphaeroides* reaction centers. In these reaction centers where an AQ is acting as Q_A, the corresponding free energy differences are 0.68 eV ($P^+Q_A^-$ – PQ_A), 0.29 eV (M – $P^+Q_A^-$) [19] and 0.19 eV (P* – P^+I^-) [14], leading to a discrepancy of 0.22 eV between the energy levels of M and P^+I^-.

Here, we have substituted the native UQ_{10} in the reaction centers from *R. sphaeroides* by three anthraquinones of low in situ redox potential and measured the corresponding energy gap between $P^+Q_A^-$ and M (ΔG_M). The ΔG_M values reflect the variations in the in situ midpoint potential of these anthraquinones measured by Woodbury et al. [14]. This suggests that M is not a state related to $P^+Q_A^-$.

2. MATERIALS AND METHODS

Native reaction centers from wild-type *R. sphaeroides* strain Y were prepared as described by Rivas et al. [17]. To remove the primary ubiquinone (Q_A), the method of Okamura et al. [18] was used with some modifications introduced by Woodbury et al. [14]. The $\Delta A_{280\ nm}/\Delta A_{802\ nm}$ ratio of the Q_A-depleted reaction centers was in the range 1.28–1.32. According to the amount of P^+ formation after a flash, about 90–95% of the reaction centers were depleted of Q_A. The remaining 5–10% displayed a decay lifetime similar to that of the native reaction centers. Anthraquinone (Fluka), 1-amino-5-chloroanthraquinone (Interchim) and 1-chloroanthraquinone (Aldrich) were dissolved in dimethyl sulfoxide. A 10-fold excess was commonly used in experiments. As compared to the UQ_{10} reconstituted reaction centers, the quantum yields of $P^+Q_A^-$ formation in 1-chloroanthraquinone, anthraquinone and 1-amino-5-chloroanthraquinone reconstituted reaction centers were 0.9, 0.95 and 0.8, respectively. 40 μM terbutryn was always present in the experiments. During experiments, the reaction centers were suspended in 10 mM Tris (pH 8), 0.03% LDAO.

Absorbance changes were recorded on a home-made flash spectrophotometer described in [19]. Exponential decomposition analysis of the kinetics was performed as in [19]. The temperature measurements were made with a thermocouple to a precision of 0.3°C.

3. RESULTS AND DISCUSSION

Arrhenius plots of the rate constant (k_{rec}) of $P^+Q_A^-$ charge recombination at pH 9 are shown in fig.1 for 1-chloroanthraquinone (a), anthraquinone (b) and 1-amino-5-chloroanthraquinone (c). As previously reported for the native reaction centers from *Rps. viridis* and *R. sphaeroides* with

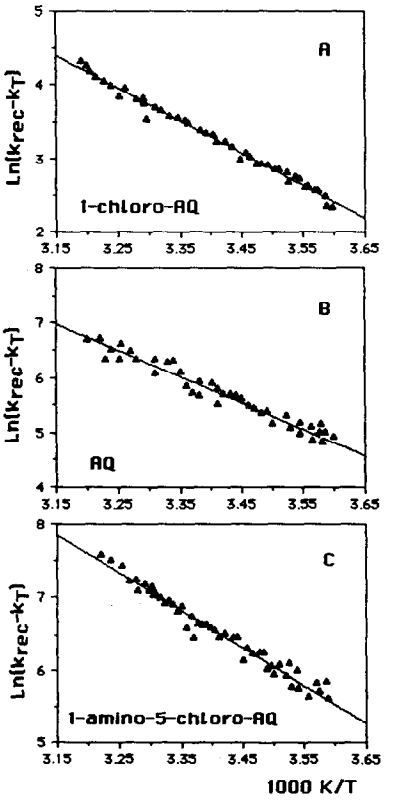

Fig.1. Arrhenius plots of k_{rec} measured at 430 nm, at pH 9, in reaction centers from *R. sphaeroides*, reconstituted with 1-chloroanthraquinone (a), anthraquinone (b) and 1-amino-5-chloroanthraquinone (c).

anthraquinone acting as Q_A, P^+ decays are non-exponential, probably due to non-equilibrium between the different protonated states of $P^+Q_A^-$ [8,10]. However, we focused here only on the ΔG_M related to the total component decays because this would not change our conclusions in terms of comparison between energy levels of the different quinones. Heterogeneity of $P^+Q_A^-$ charge recombination from 1-chloroanthraquinone and the 1-amino-5-chloroanthraquinone reconstituted reaction centers will be reported elsewhere.

Table 1 lists the activation parameters of k_{rec}. These were calculated by assuming that $k_{rec} = k_d \exp(-\Delta G_M/kT) + k_T$, as previously suggested [6,9,13] for the reaction centers where $P^+Q_A^-$ recombines via M. k_d is the rate constant for P^+I^- decay to the ground state. For reasons explained previously [6,8], we took $k_d = 2 \times 10^7\ s^{-1}$. Taking instead the measured value of

Table 1

Activation parameters of k_{rec} in anthraquinones reconstituted reaction centers from *R. sphaeroides* (E_m values from [14])

	ΔH (eV)	ΔS (meV/degree)	$-T\Delta S$ (eV)	ΔG_M (eV)	E_m (V)
1-Chloro-AQ	0.372 (±0.02)	0.132 (±0.04)	−0.039 (±0.015)	0.333 (±0.01)	−0.17
AQ	0.413 (±0.025)	0.414 (±0.05)	−0.122 (±0.02)	0.291 (±0.01)	−0.21
1-Amino-5-chloro-AQ	0.434 (±0.025)	0.624 (±0.05)	−0.184 (±0.025)	0.25 (±0.01)	−0.26

$k_d = 8 \times 10^7\ s^{-1}$ [20–22] increases the ΔG_M by 0.035 eV but does not change the differences between the ΔG_M values in table 1. k_T, the limit rate constant of recombination at low temperature, was taken as $10\ s^{-1}$ [6].

It is noteworthy that the enthalpic and entropic contributions to the ΔG_M values go in opposite directions. The ΔH_M values increase as the E_m decreases, but the high contributions of the entropic terms make the ΔG_M values effectively decrease with decreasing E_m. This phenomenon is similar to that previously observed for the two components present in native reaction centers from *Rps. viridis* [8] and in anthraquinone reconstituted reaction centers from *R. sphaeroides* [19].

As mentioned in section 1, the data available on

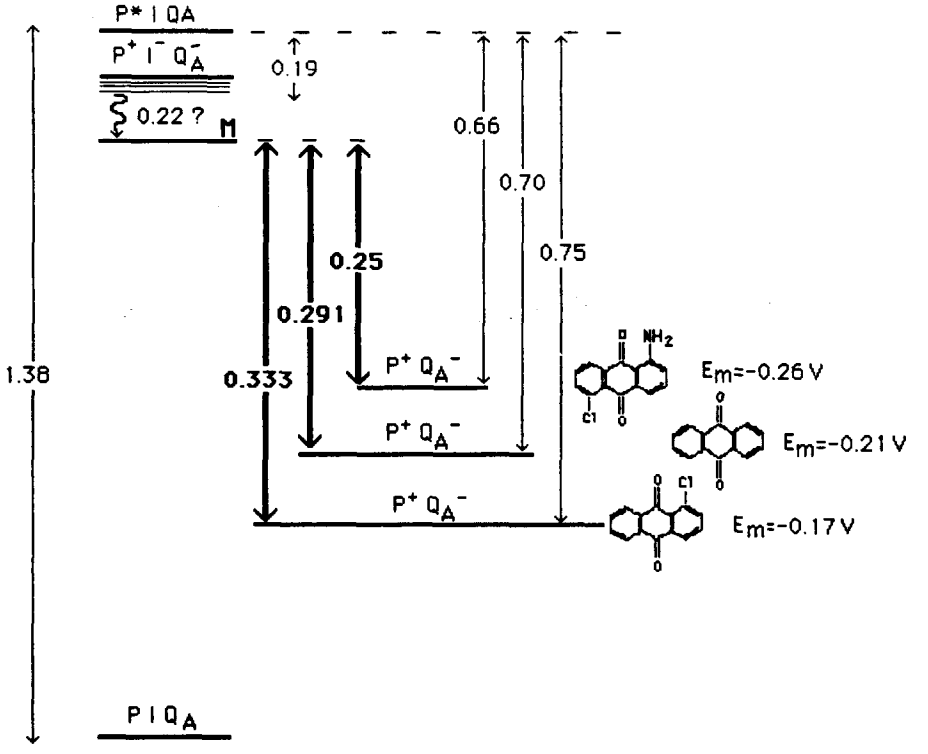

Fig.2. Scheme of the energetics of the primary separation states in reaction centers from *R. sphaeroides* reconstituted with anthraquinones. ΔG_M values measured in this paper are represented in bold-face type. Other values were determined by Woodbury et al. [14].

the energetics of AQ reconstituted reaction centers from *R. sphaeroides* are not in favor of the identification of M with the initially formed P^+I^- state. On this basis one could propose instead that if $P^+Q_A^-$ relaxes during its long lifetime (millisecond time range), the charge recombination occurs via the initial vibrational level of $P^+Q_A^-$ which could then be identified as M. In this case, one would expect to observe some relationship between the free energy levels of M and $P^+Q_A^-$. This is not what we observed.

According to the activation parameters in table 1, the differences between the ΔG_M values match the corresponding differences of the equivalent redox energies suggested by the in situ redox potential values of these anthraquinones, measured by Woodbury et al. [14]. As shown in table 1, the changes in ΔG_M ($\Delta\Delta G_M$) between 1-chloroanthraquinone and anthraquinone is 0.042 eV whereas the ΔE_m is 0.04 V; similarly, the $\Delta\Delta G_M$ between anthraquinone and 1-amino-5-chloroanthraquinone is 0.41 eV and ΔE_m amounts to 0.5 V. Thus, M is found to have a well defined free energy level, independent of the E_m of Q_A and consequently of the energy level of $P^+Q_A^-$. This suggests that M is a state independent of $P^+Q_A^-$. A relaxed form of P^+I^- appears to be the most likely candidate for being M.

Acknowledgement: I thank A.W. Rutherford for his reading of the manuscript.

REFERENCES

[1] Woodbury, N.W., Becker, M., Middendorf, D. and Parson, W.W. (1985) Biochemistry 24, 7516–7521.
[2] Martin, L.L., Breton, J., Hoff, A.J., Migus, A. and Antonetti, A. (1986) Proc. Natl. Acad. Sci. USA (1983) 957–961.
[3] Rockely, M.G., Windsor, M.W., Cogdell, R.J. and Parson, W.W. (1975) Proc. Natl. Acad. Sci. USA 72, 2251–2255.
[4] Kaufmann, K.J., Dutton, P.L., Netzel, T.L., Leigh, H.S. and Rentzepis, P.M. (1975) Science 188, 1301–1304.
[5] Shuvalov, V.A., Klevanik, A.V., Sharkov, A.V., Matveetz, Yu. and Krukov, P.G. (1978) FEBS Lett. 91, 135–139.
[6] Shopes, R.J. and Wraight, C.A. (1987) Biochim. Biophys. Acta 893, 409–425.
[7] Shopes, R.J. and Wraight, C.A. (1985) Biochim. Biophys. Acta 806, 348–356.
[8] Sebban, P. and Wraight, C.A., in preparation.
[9] Gopher, A., Blatt, Y., Schönfeld, M., Okamura, M.Y., Feher, G. and Montal, M. (1985) Biophys. J. 48, 311.
[10] Gunner, M.R., Tiede, D.M., Prince, R.C. and Dutton, P.L. (1982) in: Function of Quinones in Energy Conserving Systems (B.L. Trumpower, ed.) pp. 265–269, Academic Press, New York.
[11] Gunner, M.R., Liang, Y., Nagus, D.K., Hochstrasser, R.M. and Dutton, P.L. (1982) Biophys. J. 37, 226a (abstr.).
[12] Gunner, M.R., Robertson, D.E. and Dutton, P.L. (1986) J. Phys. Chem. 90, 3183–3195.
[13] Kleinfeld, D., Okamura, M.Y. and Feher, G. (1985) Biophys. J. 48, 849–852.
[14] Woodbury, M.W., Parson, W.W., Gunner, M.R., Prince, R.C. and Dutton, P.L. (1986) Biochim. Biophys. Acta 851, 16–22.
[15] Carithers, R.P. and Parson, W.W. (1975) Biochim. Biophys. Acta 387, 194–211.
[16] Hörber, J.K.H., Göbel, W., Ogrodnik, A., Michel-Beyerle, M.E. and Cogdell, R.J. (1986) FEBS Lett. 198, 268–272.
[17] Rivas, E., Husson-Reiss, F. and Le Maire, M. (1980) Biochemistry 19, 2943–2950.
[18] Okamura, M.Y., Isaacson, R.A. and Feher, G. (1975) Proc. Natl. Acad. Sci. USA 72, 3492–3495.
[19] Sebban, P., (1988) submitted.
[20] Schenck, C.C., Blankenship, R.E. and Parson, W.W. (1982) Biochim. Biophys. Acta 680, 44–59.
[21] Chidsey, C.E.D., Kirmaier, C., Holten, D. and Boxer, S.G. (1984) Biochim. Biophys. Acta 766, 424–437.
[22] Budil, D.E., Kolaczkowski, S.V. and Norris, J.R. (1987) in: Progress in Photosynthesis Research (Biggins, J. ed.) vol.1, pp. 25–27, Martinus Nijhoff, Dordrecht.