Biochimica et Biophysica Acta, 724 (1983) 317–322
Elsevier

BBA 41351

DIFFERENT TEMPERATURE DEPENDENCIES OF THE CHARGE RECOMBINATION REACTION IN PHOTOREACTION CENTERS ISOLATED FROM DIFFERENT BACTERIAL SPECIES

TED MAR, CHRISTIAN VADEBONCOEUR * and GABRIEL GINGRAS

Département de Biochimie, Université de Montréal, C.P. 6128, Montréal, Québec H3C 3J7 (Canada)

(Received May 3rd, 1983)

Key words: Reaction center; Bacterial photosynthesis; Charge recombination; Temperature dependence; Electron transfer

We compared the temperature dependency of the rate of the charge recombination reaction in photoreaction centers isolated from *Ectothiorhodospira* sp. and from *Rhodospirillum rubrum* G9. We also examined the temperature dependency of the bandwidth and peak wavelength of their far-red absorption band. In both preparations, the peak wavelength and the bandwidth vary monotonically with temperature between 80 and 300 K. However, the rate of the charge recombination reaction has a quite different temperature dependency. In the preparation from *R. rubrum,* the reaction is accelerated 5-fold in a typical sigmoidal fashion as the temperature is lowered from 300 to 80 K. In the preparation from *Ectothiorhodospira* sp., the reaction is accelerated monotonically only about 1.5-fold in the same temperature range. At temperatures below 100 K, the rates are similar in the two preparations. We interpret the temperature dependency of the charge recombination reaction in terms of an activationless electron-transfer model formulated by Jortner (Jortner, J. (1980) Biochim. Biophys. Acta 394, 193–230). The minimal model provides a good fit for the temperature dependency of charge recombination in the preparation from *Ectothiorhodospira* sp. However, to fit the temperature dependency of the *R. rubrum* preparation with the same model, we must further postulate that the electronic coupling factor varies with temperature in this preparation. We find that, in both preparations, the temperature dependency of the far-red absorption bandwidth is consistent with the assumption that similar vibrational modes are involved in electron transfer and in electronic excitation.

Introduction

The photoreaction center is the site of a photochemical charge separation reaction between a primary electron donor and a primary electron acceptor. In the isolated photoreaction center, the charges recombine in a dark reaction called the back-reaction. This back-reaction has a peculiar temperature dependency in the bacterial photoreaction center preparations so far studied. For instance, in preparations from *Rhodopseudomonas sphaeroides* and from *Rhodospirillum rubrum* the back-reaction shows first-order kinetics with half-times of about 80 ms at room temperature and of about 20 ms at temperatures below 150 K [1–6]. The rate is temperature independent below 150 K and this indicates that quantum-mechanical tunnelling plays a role in that reaction. Clayton [7] has provided evidence that this peculiar temperature dependence may be attributed to one or more phase transitions involving water.

This unusual temperature dependency has led to many speculations concerning the mechanism of the back-reaction. Hsi and Bolton [4], Hales [8] and Hoff [9] have speculated that the molecular

---

* Present address: Ecole de Médecine Dentaire, Université Laval, Québec, Canada.

distance between the primary acceptor and the primary donor increases with increasing temperature. Hopfield [10], Sarai [11], Kakitani and Kakitani [12], Jortner [13] and Kuznetsov and Ulstrup [14] have proposed that electron transfer occurs between molecules more or less rigidly held by the protein matrix and that the temperature dependency is determined by the vibronic coupling of the individual molecules and by the low-frequency vibrational mode of the protein. However, with the exception of that of Kakitani and Kakitani [12], recent theories on vibronically coupled tunnelling have been unsuccessful in predicting the more than 4-fold increase in electron transfer rate observed between 150 and 300 K for photoreaction centers from *Rps. sphaeroides* and *R. rubrum.*

In the present work, we have compared the temperature dependency of the back-reaction in photoreaction centers isolated from *R. rubrum* and from *Ectothiorhodospira* sp. We found that the latter preparation has a back-reaction whose temperature dependency can be predicted by a simple model such as the one proposed by Jortner [13]. Moreover, the temperature dependency of this back-reaction is very similar to that of dehydrated preparations from *Rps. sphaeroïdes* [7].

Materials and Methods

R. rubrum G9 was grown as described before [15]. *Ectothiorhodospira* sp., a gift from Dr. E. Newman, Concordia University, Montréal, Québec, was grown according to the method of Bognar et al. [16]. Photoreaction center from *R. rubrum* was extracted by solubilization with lauryldimethyamine *N*-oxide and purified according to the method of Vadeboncoeur et al. [17]. The photoreaction center of *Ectothiorhodospira* sp. was obtained by a similar method (Lefebvre, S., Cloutier, Y., Picorel, R. and Gingras, G., unpublished data). The photoreaction center preparations were dispersed in 10 mM Tris-HCl (pH 7.5)/0.1% Triton X-100 containing 50% (v/v) glycerol.

The absorbance changes were observed with a single-beam spectrophotometer [18]. The actinic-light flash was either from a dye laser (Candela SL-66) or from a xenon flashlamp (Braun F240 LS) with flash duration of 100 ns and 1–3 ms, respectively. The same back-reaction rate was found with either of those sources which, therefore, were used interchangeably. For experiments with the dye laser, the exciting wavelength was 605 nm (rhodamine 6G in ethanol) and the measuring wavelength was 870 nm. A second monochromator plus selected cutoff filters were placed between the excitation monochromator and the sample chamber to block scattered light from the laser. In experiments with the xenon flash, excitation was in the far-red region as defined by Schott RG 9 cutoff filters and the measuring wavelength was 605 nm. The measurements were averaged with a Biomation 102 signal averager. All measurements were performed in a Joule-Thomson cryostat (Air Products Corp.). Absorption spectra were determined in a Cary 14R spectrophotometer.

Results and Discussion

Fig. 1a shows the absorption spectrum of the photoreaction center from *Ectothiorhodospira* sp. at 77 and 286 K. The 286 K spectrum has interesting features when compared with the absorption spectrum from *R. rubrum* G9 (Fig. 1b): the two far-red peaks of bacteriochlorophyll are shifted, one to the blue (799 nm instead of 802 nm) and the other to the red (883 nm instead of 865 nm). The 753 nm peak, attributed to bacteriopheophytin, is also slightly red shifted with respect to the preparation from *R. rubrum* (Fig. 1b). On lowering the temperature to 77 K the absorption bands of both preparations are narrowed and become more intense. At the same time, the far-red band is shifted from 865 to 888 nm in the preparation from *R. rubrum* (Fig. 1b) and from 883 to 905 nm in the preparation from *Ectothiorhodospira* sp. (Fig. 1a). The other bands show no significant shift.

In the photoreaction center from *Ectothiorhodospira* sp., the charge recombination kinetics after a single short exciting flash are multiphasic at room temperature. In the presence of *o*-phenanthroline, a known inhibitor of electron transfer from primary to secondary acceptor [19], the charge recombination reduces to first-order kinetics with a lifetime of 37 ms. At 77 K, the signal is still reversible even after many short single flashes. Under those conditions, the decay lifetime is 24 ms. This effect of *o*-phenanthroline and the re-

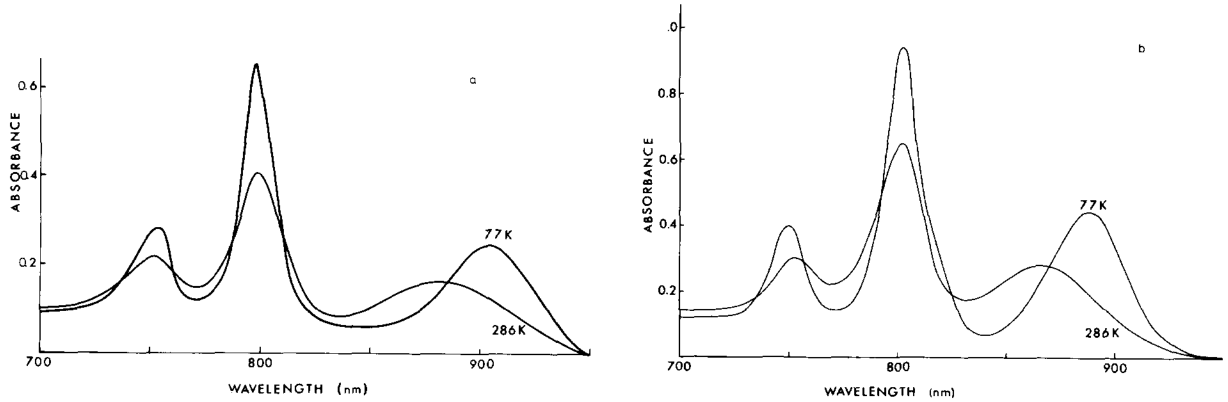

Fig. 1. Absorption spectra of photoreaction centers isolated from *Ectothiorhodospira* sp. (a) and from *R. rubrum* G9 (b). The preparations were in 10 mM Tris-HCl (pH 7.5) containing 50% glycerol and 0.02% (v/v) lauryldimethylamine *N*-oxide.

versibility of the signal at 77 K are typical of a true charge recombination reaction between the primary donor and the primary acceptor. Next, the exponential lifetime of this primary back-reaction was measured as a function of temperature between 77 and 286 K in photoreaction centers from *R. rubrum* G9 and from *Ectothiorhodospira* sp. (Fig. 2). In photoreaction centers from *R. rubrum*, we observe the typical [1–5] sigmoidal temperature dependency with a reaction lifetime diminishing by a factor of nearly 5 when lowering the temperature from 286 to 77 K. In photoreaction centers from *Ectothiorhodospira* sp., the temperature dependency, while also sigmoidal, is only slight: the reaction lifetime is decreased by a factor of approx. 1.5 between 286 and 77 K.

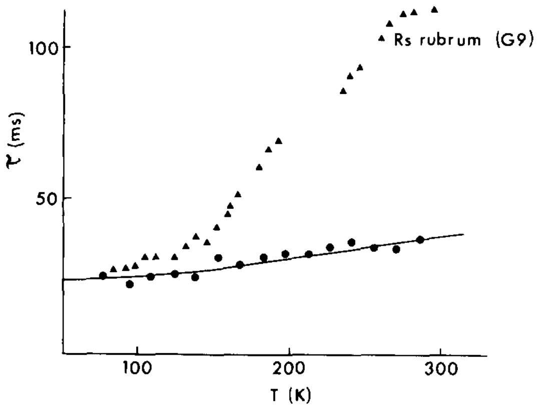

Fig. 2. Temperature dependency of the charge recombination reaction in photoreaction centers from *R. rubrum* G9 and from *Ectothiorhodospira* sp. The continuous line in the latter case was calculated from Eqns. 1a and 1b. The exponential lifetime of the reaction was followed by measuring the change of absorbance following flash-induced bleaching. The photoreaction center preparations were suspended in 1 mM *o*-phenanthroline.

Recent theories on vibronically coupled electron tunnelling accurately predict the temperature dependency of the electron-transfer rate between cytochrome *c* and the primary electron donor of the photoreaction center [10–14,20]. However, it seems that the peculiar temperature dependency of the back-reaction cannot be fitted by minimal models [13] and that some additional parameters such as vibrational frequency changes must be taken into account [12]. This raises the possibility that the simpler models may be too oversimplified to explain such complicated phenomena as the back-reaction. In this work, we have observed a different type of temperature dependency in a photoreaction center from *Ectothiorhodospira* sp. We will show that this temperature dependency can be fitted exactly by a minimal model such as the one proposed by Jortner [13].

The main features of this model are that: (a) the electron donor and acceptor are spatially fixed, (b) electron transfer is described as a transition between two one-dimensional nuclear potential surfaces corresponding to the initial and final states of the entire system, (c) a single mode approximation is assumed in which a single molecular vibration (characteristic frequency) undergoes appreciable configurational change during electron transfer. As a consequence, the nuclear potential surfaces are displaced with respect to one another, (d) the back-reaction is an activationless electron-transfer process [21] and, therefore, intersection of the initial and final nuclear potential surfaces occurs at a minimum of the former.

According to this model, the rate of electron transfer is shown by Jortner (Eqns. V.2 and V.3 of Ref. 13) to be

$$W_0 = \frac{2\pi}{\hbar}|V|^2 \frac{1}{\omega(2\pi S)^{1/2}} \quad (kT \ll \hbar\omega) \qquad (1a)$$

$$W = W_0\left(\frac{\hbar\omega}{2kT}\right)^{1/2} \quad (kT \gg \hbar\omega) \qquad (1b)$$

where W_0 and W are the rate of electron transfer at low and at high temperatures, respectively; V the electronic coupling factor which depends on the separation and relative orientation of the donor and acceptor; S the electronic-vibrational coupling factor which is governed by the horizontal displacement of the minimum of the initial and final nuclear potential surfaces; ω the characteristic frequency of the nuclear mode which undergoes a configurational change during electron transfer; T the temperature and k and $\hbar$, respectively, Boltzmann's constant and Planck's constant divided by 2π. The theory for an activationless reaction predicts a temperature-independent rate for temperatures smaller than $\hbar\omega/2k$ and a $T^{-1/2}$ dependence of the rate for temperatures above $\hbar\omega/2k$.

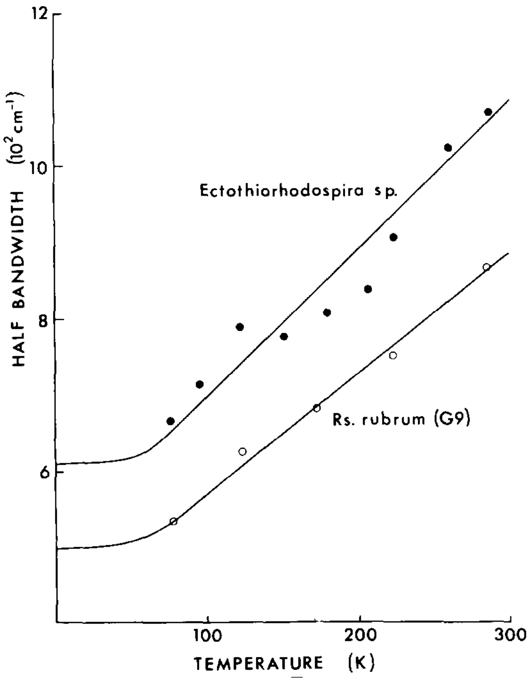

Fig. 3. Bandwidth temperature dependency of the far-red band in photoreaction centers from *R. rubrum* G9 and from *Ectothiorhodospira* sp. The points are experimental values whereas the continuous lines are plotted according to Eqns. 2a and 2b. Experimental conditions were as in Fig. 1.

Fig. 2 shows a plot of the experimental temperature dependency of the back-reaction kinetics in photoreaction centers from *Ectothiorhodospira* sp. (points) compared to a theoretical curve (continuous line) calculated from Eqn. 1. This curve was obtained using a characteristic frequency, ω, of 167 cm^{-1}. The fit is seen to be excellent. As also shown on Fig. 2, Eqn. 1 does not predict the temperature dependency of the preparation from *R. rubrum* where the rate is increased approx. 5-fold between 150 and 300 K. Interestingly, below 100 K the rate of electron transfer, W_0, tends to the same value in the two preparations (Fig. 2). This indicates that, at those low temperatures, the rate of electron transfer of both bacterial preparations can be described by the same values of the three parameters V, S and ω.

S, the electronic-vibrational coupling factor for activationless electron transfer, can be calculated from the relationship $S = \Delta E/\hbar\omega$ [13], where ΔE is the electronic energy gap between the initial and final electronic states. ΔE has been estimated to be 6000 cm^{-1} for the back-reaction [13]. S can then be calculated to be 36 for the back-reaction in the photoreaction center from *Ectothiorhodospira* sp.

An independent rough estimate of the characteristic frequency of electron transfer can be obtained from the characteristic frequency of absorption, since similar vibrations are expected to be involved in both processes. Indeed, the broadening of optical spectral lines can be disucssed using the same formalism as for electron transfer. With this formalism, Williams and Hebb [22] and later Markham [23] have shown that the bandwidth of a Gaussian absorption band (Eqns. 7.7b, 11.3 and 11.4 of Ref. 23) is given by

$$H_0 = \sqrt{8\ln 2}\,(\hbar\omega_a)S_a^{1/2} \quad (kT \ll \hbar\omega_a) \qquad (2a)$$

$$H = H_0\left(\coth\left(\frac{\hbar\omega_a}{2kT}\right)\right)^{1/2} \quad (kT \gg \hbar\omega_a) \qquad (2b)$$

where H_0 and H are the bandwidths at low and high temperature, respectively. S_a and ω_a are defined in the same way as in the electron-transfer process. The theory predicts that the bandwidth is constant for temperatures lower than $h\omega/2k$ and follows a $\coth^{1/2}(T^{-1})$ dependence for temperatures higher than $h\omega/2k$. Fig. 3 compares the experimental bandwidth temperature dependency in photoreaction centers from *Ectothiorhodospira* sp. and from *R. rubrum* with curves calculated with Eqn. 2. A good fit is obtained for both preparations with a characteristic frequency of 139 cm^{-1}. This value is different but not too far from the characteristic frequency of 167 cm^{-1} calculated above for electron transfer.

The value of S_a, the electronic-vibrational coupling factor for the absorption process, that will best fit the experimental bandwidth temperature dependency (Fig. 3 and Eqn. 2) is 18.5 and 15.2 for the preparations from *Ectothiorhodospira* sp. and from *R. rubrum,* respectively. This is clearly lower than the value of 36 that was calculated above for S, the electronic-vibrational coupling factor for electron transfer. However, this difference is not unexpected. Since the removal of an electron causes a larger perturbation than the optical excitation of an electron, it follows that S, the factor which represents the distortion of the equilibrium configuration of the nuclear potential surface of the initial and final systems, whould be larger in electron transfer than in absorption.

From the above results, the bandwidth temperature dependency behavior of the photoreaction center preparations from *Ectothiorhodospira* sp. and from *R. rubrum* can be described by similar values of S_a and ω_a. Moreover, the fact that ω and ω_a are similar for the preparation from *Ectothiorhodospira* sp. is consistent with the hypothesis that similar vibrational modes are involved in absorption and in activationless electron transfer. Consequently, one might expect that the preparations from *R. rubrum* and *Ectothiorhodospira* sp. would have similar values of S and ω. This should entail similar rates for the back reaction in these two preparations, if Jortner's model is a good description of both. Experimentally, this prediction is only verified for temperatures below 100 K (Fig. 2). Indeed, as the temperature decreases, the rate of the back-reaction decreases much more rapidly in the photoreaction center from *R. rubrum* than in the preparation from *Ectothiorhodospira* sp. To fit the *R. rubrum* case, a further assumption has to be introduced into Jortner's model, namely, that in this case the electronic coupling factor, V, varies with temperature. This would imply that in the photoreaction center of *R. rubrum,* the slowing down of the back-reaction at higher temperatures would probably be due to a change in the relative orientation or in the separation between the donor and acceptor molecules as has been proposed by others [4,8,9]. We cannot, for the moment, distinguish between these possibilities. We can only add that the similar temperature dependencies of the peak wavelength in the two preparations (Fig. 4) does not indicate that their primary electron donors are undergoing different environmental changes.

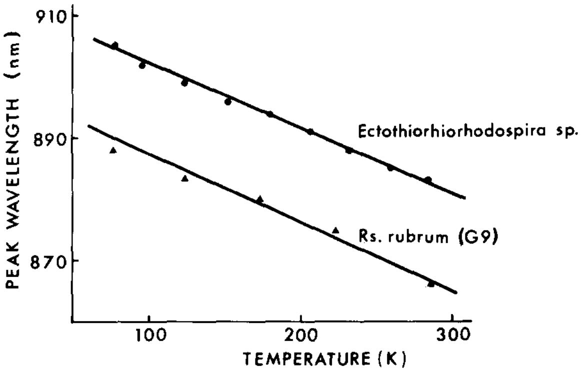

Fig. 4. Temperature dependency of the far-red band in photoreaction centers from *Ectothiorhodospira* sp. and *R. rubrum* G9. Experimental conditions were as in Fig. 1.

The temperature dependency of the back-reaction in the preparation from *Ectothiorhodospira* sp. is remarkably similar to that observed by Clayton [7] in dehydrated photoreaction centers from *Rps. sphaeroides*. One might suggest that, in both the dehydrated preparation from *Rps. sphaeroides* and in the untreated preparation from *Ectothiorhodospira* sp., the distance and the relative orientation of the primary donor and of the primary acceptor are independent of temperature.

In summary, we have shown that the peculiar temperature dependency of the rate of the back-reaction is not common to all bacterial photoreaction centers. We have interpreted our results by a minimal electron-transfer model proposed by

Jortner [13]. If the model is correct, we should expect that the electron donor and acceptor are spatially fixed in *Ectothiorhodospira* sp. and that they are less rigidly held in *R. rubrum.* This needs to be verified experimentally.

Acknowledgements

This research was supported by grants from the National Sciences and Engineering Research Council Canada and by the Ministère de l'Education du Québec.

References

1 Parson, W.W. (1967) Biochim. Biophys. Acta 131, 154–172
2 Clayton, R.K. and Yau, H.F. (1972) Biophys. J. 12, 867–881
3 Loach, P.A., Kung, M. and Hales, B.J. (1973) Ann. N.Y. Acad. Sci. 224, 297–319
4 Hsi, E.S.P. and Bolton, J.R. (1974) Biochim. Biophys. Acta 347, 126–133
5 Romjin, J.C. and Amesz, J. (1976) Biochim. Biophys. Acta 423, 164–173
6 McElroy, J.D., Feher, G. and Mauzerall, D.C. (1969) Biochim. Biophys. Acta 172, 180–183
7 Clayton, R.K. (1978) Biochim. Biophys. Acta 504, 255–264
8 Hales, B.J. (1976) Biophys. J. 16, 471–480
9 Hoff, A.J. (1979) Phys. Rep. 54, 75–200
10 Hopfield, J.J. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 3640–3644
11 Sarai, A. (1980) Biochim. Biophys. Acta 589, 71–83
12 Kakitani, T. and Kakitani, H. (1981) 635, 498–514
13 Jortner, J. (1980) Biochim. Biophys. Acta 594, 193–230
14 Kuznetsov, A.M. and Ulstrup, J. (1981) Biochim. Biophys. Acta 636, 50–57
15 Noël, H., Van der Rest, M. and Gingras, G. (1972) Biochim. Biophys. Acta 275, 219–230
16 Bognar, A., Desrosiers, L., Libman, M. and Newman, E.B. (1982) J. Bacteriol. 152, 706–713
17 Vadeboncoeur, C., Noël, H., Poirier, L., Cloutier, Y. and Gingras, G. (1979) Biochemistry 18, 4301–4307
18 Mar, T., Picorel, R. and Gingras, G. (1983) Biochim. Biophys. Acta 682, 354–363
19 Parson, W.W. (1970) Biochim. Biophys. Acta 205, 232–245
20 DeVault, D.C. (1980) Q. Rev. Biophys. 13, 387–564
21 Hopfield, J.J. (1979) in Tunnelling in Biological Systems (Chance, B., DeVault, D.C., Frauenfelder, H., Marcus, R.A., Schrieffer, J.R. and Sutin, N., eds.), pp. 417–432, Academic Press, New York
22 Williams, F.E. and Hebb, M.H. (1951) Phys. Rev. 84, 1181–1183
23 Markham, J.J. (1959) Rev. Mod. Phys. 31, 956–989.