

**Acknowledgment.** This work was supported by the Office of Naval Research.

### References and Notes

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## The Question of Dispersive Kinetics for the Initial Phase of Charge Separation in Bacterial Reaction Centers

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(Received: June 17, 1992)

Recently, femtosecond time domain data have raised the possibility that the kinetics of the initial phase of charge separation could be dispersive due to the glasslike structural heterogeneity of proteins. Guided by spectral hole burning data, we have derived simple theoretical rate expressions which allow the question posed in the title to be explored.

The X-ray diffraction determinations of the structures of the reaction centers (RC) of the purple bacteria *Rhodospseudomonas viridis*<sup>1</sup> and *Rb. sphaeroides*<sup>2,3</sup> have led to a burgeoning of experimental and theoretical studies of the initial phase of charge separation which originates from the lower Q<sub>y</sub> dimer state (P\*) of the special pair. It is generally taken that P\*B<sup>-</sup>H figures importantly in the formation of the P\*BH<sup>-</sup> charge-separated state. (Here B and H denote the active bacteriochlorophyll and bacteriopheophytin monomers, respectively.) Discussion continues unabated on the relative importance of the superexchange (one-step) and two-step mechanisms for the production of P\*BH<sup>-</sup> where, in the latter, P\*B<sup>-</sup>H serves as a real intermediate state. Zinth and co-workers<sup>4-6</sup> have interpreted their femtosecond transient absorption data in terms of the two-step mechanism. The biphasic time constants for the first and second steps of *Rb. sphaeroides* at 300 and 25 K are ( $\tau_1, \tau_2$ ) = (3.5 ± 0.4, 1.2 ± 0.3) and (1.4 ± 0.3, 0.3 ± 0.15) ps, respectively.<sup>6</sup> These workers emphasize that judicious selection of the probe wavelength was critical to the success of the experiments. Chan et al.<sup>7</sup> have also interpreted their 300 K femtosecond transient absorption data in terms of the two-step mechanism. However, Vos et al.<sup>8</sup> state that the two-step mechanism is inconsistent with their femtosecond results. Kirmaier and Holten have argued against the two-step mechanism and interpreted their temperature- and wavelength-dependent data in terms of structural heterogeneity.<sup>9</sup>

Adding to the complexity of the story is the finding that<sup>8</sup> the stimulated emission from P\* of *Rb. sphaeroides* (R26 mutant) at room temperature is nonexponential. The data could be fit by a biexponential with decay times of 2.9 ps (65%) and 12 ps (35%). The existence of a long-lived component has been confirmed by spontaneous emission studies.<sup>10-12</sup> In the last of these references, ~50-fs time resolution was achieved using a nonlinear upconversion scheme. For R26, *Rhodobacter capsulatus*, and several of its mutants it was observed that an increase in the amplitude of the long-lived component could be positively correlated with the fwhm of the P band at 4.2 K.<sup>12</sup> Furthermore, it appears that

the decay times of the short-lived components are in quite good agreement with the P\* decay times determined earlier on the basis of stimulated emission.

Given that spectral hole burning had already unraveled the underlying structure of the absorption band of P and proven that<sup>13,14</sup> both P870 and P960 of *Rb. sphaeroides* (R26) and *Rps. viridis* suffer from significant site inhomogeneous broadening ( $\Gamma_1$ ), Table I, it is timely to consider whether the "glasslike" structural heterogeneity of proteins, which gives rise to  $\Gamma_1$ , could be responsible for the aforementioned nonexponential decay of P\*. That is, the nonexponentiality could be due to dispersive kinetics stemming from a distribution of values for the energy gap(s) relevant to the primary charge separation process. The existence of  $\Gamma_1$  is proof positive of random fluctuations in structure from RC to RC for a given complex. However, its existence does not prove that there are distributions for the energy gaps. Importantly, however, spectral hole burning data have proven that there is an absence of correlation between the Q<sub>y</sub> state energies of the accessory chlorophyll and active pheophytin of the RC of photosystem II.<sup>15</sup> Given that proteins are glasslike, we are of the opinion that this absence of correlation should be generally true.

It is our purpose here to argue that distributions of relevant energy gaps are unlikely to give rise to measurable dispersive kinetics via decay curves even at liquid helium temperatures. This is a consequence of the fact that the linear electron-phonon coupling is too strong. Nevertheless, the calculations indicate that variations in  $\Gamma_1$  from sample quality, host medium, etc., could lead to significantly different decay kinetics as measured at liquid helium temperatures. Insofar as the aforementioned nonexponential decay behavior of P\* at room temperature is concerned, we conclude that it must be due to something else, e.g., a distribution of values for relevant electron-exchange matrix elements associated with structural heterogeneity.

With reference to Table I and refs 13 and 14, where these data are discussed in detail, we emphasize that the overall linear electron-phonon coupling for the P\* ← P optical transition is

TABLE I: Optical Electron-Photon Coupling and Inhomogeneous Broadening Parameter Values<sup>a</sup>

	$\bar{\omega}$ (cm <sup>-1</sup> )	$S(0\text{ K})$ ( $S_0$ )	$\omega_{sp}$ (cm <sup>-1</sup> )	$S_{sp}(0\text{ K})$ ( $S_{0,sp}$ )	$\sigma$ (cm <sup>-1</sup> )	$\sigma_{sp}^b$ (cm <sup>-1</sup> )	$\Gamma_1^c$ (cm <sup>-1</sup> )	$\sum S_i \omega_i$ (cm <sup>-1</sup> )
P960	25	2.1	135	1.1	20	25	120	200
P870 (R26)	30	2.2	120	1.5	20	25	170	240

<sup>a</sup>Data from refs 13 and 14. <sup>b</sup>Hole burning results lead to a  $\sim 100$ -fs relaxation time for the special pair marker mode which yields a  $50\text{-cm}^{-1}$  homogeneous width. <sup>c</sup>The samples used in ref 13 yielded fwhm values for P960 and P870 of  $420$  and  $470\text{ cm}^{-1}$  at  $4.2\text{ K}$ . To our knowledge, sharper profiles have *not* been reported.

TABLE II: Parameter Values for Calculations

	$\bar{\omega}$ (cm <sup>-1</sup> )	$S(0\text{ K})$	$\omega_{sp}$ (cm <sup>-1</sup> )	$S_{sp}(0\text{ K})^a$	$\sigma$ (cm <sup>-1</sup> )	$\sigma_{sp}$ (cm <sup>-1</sup> )	$\Gamma$ (cm <sup>-1</sup> )	$\Omega_0$ (cm <sup>-1</sup> )
P960	30	7.0	135	1.1	20	25	85	300
P870 (R26)	30	6.0	120	1.5	20	25	120	300

<sup>a</sup>Chosen the same as in Table I.

strong. This coupling involves protein "phonons" of mean frequency  $\sim 30\text{ cm}^{-1}$  ( $\bar{\omega}$ ) and a special pair intermolecular marker mode ( $\omega_{sp}$ ). The total optical reorganization energy is  $\sim 200$  and  $\sim 240\text{ cm}^{-1}$  for P960 and P870, respectively. Since the charge-transfer character of  $P^*$  is only  $\sim 0.3$ ,<sup>16</sup> it is reasonable to assume that the coupling for the  $P^*BH \rightarrow P^*B^-H$  or  $P^*BH^-$  electron-transfer processes is stronger than for  $P^* \leftarrow P$ . In what follows we denote  $P^*BH$  as D and  $P^*B^-H$  or  $P^*BH^-$  as A. In the strong electron-phonon coupling limit the rate constant for a single RC can be approximated as<sup>17</sup>

$$k_{DA} = 2\pi V^2 [2\pi S(\sigma^2 + \bar{\omega}^2)]^{-1/2} \exp[-(\Omega - S_0\bar{\omega})^2 / 2S(\sigma^2 + \bar{\omega}^2)] \quad (1)$$

where  $\Omega = \omega_D - \omega_A$ ,  $S = S_0 \coth(\hbar\bar{\omega}/2kT)$ ,  $S_0$  is the 0 K Huang-Rhys factor for the protein phonons,  $V$  is the electronic coupling matrix element, and  $2\sigma$  is the width of the one-phonon profile associated with the nuclear factor of the Fermi-Golden rule rate expression.<sup>18</sup> Equation 1 is readily generalized to include the special marker mode which we observe is likely to be "silent" for the  $P^*B^-H \rightarrow P^*BH^-$  transfer of the two-step mechanism. For simplicity, we have taken the one-phonon profile to be a Gaussian.<sup>18</sup> For a Gaussian distribution of  $\Omega$  values centered at  $\Omega_0$ , the average value for the rate is

$$\langle k_{DA} \rangle = 2\pi V^2 [2\pi(\Gamma^2 + S(\sigma^2 + \bar{\omega}^2))]^{-1/2} \exp[-(\Omega_0 - S_0\bar{\omega})^2 / 2(\Gamma^2 + S(\sigma^2 + \bar{\omega}^2))] \quad (2)$$

where  $2\Gamma$  is the width of the normal distribution for  $\Omega$ . The time dependence of the  $P^*$  population is

$$P^*(t) = P^*(t=0) \int d\Omega f_{\Omega} \exp(-k_{DA}(\Omega)t) \quad (3)$$

where  $k_{DA}(\Omega)$  is given by eq 1 and  $f_{\Omega}$  is the Gaussian distribution function. Equations 1 and 2 are more informative than the usual "Einstein-stick" expression<sup>19</sup> for  $k_{DA}$  since they take into account the homogeneous line shape of the nuclear factor. We emphasize that we do not take into account here the possibility of a distribution of  $V$  values since our intent is only to explore dispersive kinetics arising from a distribution of energy gap values. It should be apparent that dispersive kinetics from the latter distribution will only be important when  $\Gamma^2 > S(\sigma^2 + \bar{\omega}^2)$  or when the marker mode is included,  $\Gamma^2 > [S(\sigma^2 + \bar{\omega}^2) + S_{sp}(\sigma_{sp}^2 + \omega_{sp}^2)]$ . A key point is that the  $S$ 's carry a temperature dependence ( $S_{sp} = S_{sp}(0\text{ K}) \coth(\hbar\omega_{sp}/2kT)$ ).

In what follows, we assume that the  $\Gamma_1$  values for the absorption bands from the ground state to the charge-transfer states are the same as those observed for the  $P^* \leftarrow P$  bands. In the absence of correlation (vide supra), this means that  $2\Gamma = 2^{1/2}\Gamma_1$  where  $\Gamma_1$  is the inhomogeneous broadening of the  $P^* \leftarrow P$  absorption band. The values of the parameters used in the calculations for *Rb. sphaeroides* and *Rps. viridis* are given in Table II. All, except for the  $D \rightarrow A$  reorganization energy and  $D-A$  adiabatic electronic energy gap, obtain from the hole burning data. For the former we use a value of  $360\text{ cm}^{-1}$  based on the very accurately determined reorganization energy (due to phonons) of the  $S_1 \leftarrow S_0$  pure

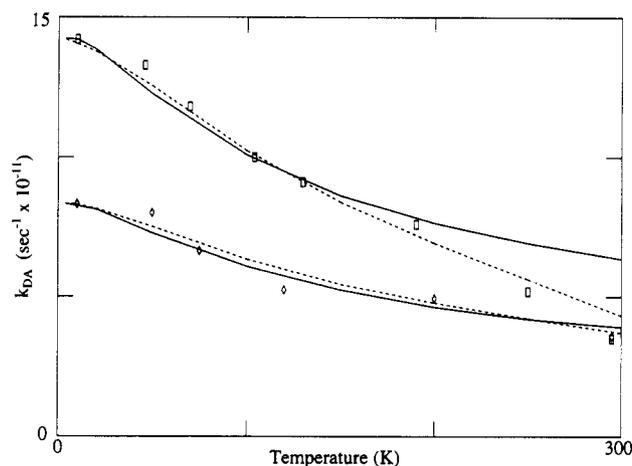


Figure 1. Rate of primary electron transfer as a function of temperature: rectangles, *Rps. viridis*; diamonds, *Rb. sphaeroides*; solid line, theoretical fit (eq 2) with parameters of Table II; dotted lines, theoretical fit including a thermal shift of  $\Omega_0$ . The experimental data are from ref 25.

charge-transfer (CT) transition of the anthracene-pyromellitic acid dianhydride (PMDA) crystal.<sup>20</sup> This is not much larger than the optical reorganization of the  $P^* \leftarrow P$  transition. However, the dipole moment change of  $\sim 11\text{ D}$  for anthracene-PMDA<sup>20</sup> is not much larger than those measured for the  $P^* \leftarrow P$  transition.<sup>21-23</sup> We hasten to add that a larger value for the  $D \rightarrow A$  reorganization energy would only strengthen our conclusion that a distribution of values for relevant energy gap should not lead to dispersive kinetics. The calculations are intended to be relevant to either the  $P^*BH \rightarrow P^*B^-H$  or  $P^*BH^-$  (superexchange) processes. For both we use an  $\omega_D - \omega_A$  gap of  $300\text{ cm}^{-1}$  for mediation by the protein phonons and special pair marker mode. Although this gap for the latter process is  $\approx 2000\text{ cm}^{-1}$ ,<sup>24</sup> we note that, with our values for the linear electron-phonon coupling parameters, it would be imperative to take into account the high-frequency intramolecular modes of B and H to achieve a maximum rate. Inclusion of these modes would reduce the effective gap for the phonons and marker mode to a few hundred  $\text{cm}^{-1}$ .

Inspection of Table II reveals that the observation of dispersive kinetics for the  $D \rightarrow A$  electron-transfer process would be most likely for *Rb. sphaeroides* at liquid helium temperatures. We have numerically evaluated eq 3 for  $P^*(t)$  and find that the dispersion for *Rb. sphaeroides* at low temperature is too slight to be observable by current ultrafast time domain systems (results not shown). In consideration of this finding it should be noted that the "homogeneous width" of the nuclear Franck-Condon factor  $2[(S(\sigma^2 + \bar{\omega}^2) + S_{sp}(\sigma_{sp}^2 + \omega_{sp}^2))]^{1/2}$  at  $4.2\text{ K}$  is  $350\text{ cm}^{-1}$ , which is larger than  $2\Gamma = 240\text{ cm}^{-1}$ . At  $300\text{ K}$  the homogeneous width is  $870\text{ cm}^{-1}$ .

The degree to which the above reasoning and result are reasonable may be judged somewhat by the ability of eq 2 to account for the temperature dependence of  $P^*$ 's lifetime. In Figure 1 the

rectangles and diamonds are the experimental data points for P960\* and P870\* of *Rps. viridis* and *Rb. sphaeroides*, respectively.<sup>25</sup> The experimental uncertainty is about  $\pm 20\%$ . The solid curves are the theoretical fits obtained with the parameter values given in Table II. They are as good as those given by Bixon and Jortner,<sup>19</sup> who use a single mean phonon frequency (a "free" parameter) of  $\sim 80 \text{ cm}^{-1}$ , which is close to the mean of the experimentally measured protein phonon and marker mode frequencies. As in their work, our fit for *Rps. viridis* for  $T > \sim 200 \text{ K}$  is unsatisfactory. Rather than ascribing this to a temperature contraction of the protein peculiar to *Rps. viridis*, we consider the possibility that the stronger temperature dependence for *Rps. viridis* is due to temperature dependence of  $\Omega_0$  (eq 2). That P870\* and P960\* red shift by  $\sim 300 \text{ cm}^{-1}$  as the temperature is reduced from room to liquid helium temperatures is well established.<sup>26</sup> Unfortunately, the temperature dependences for the P\*B<sup>-</sup>H and P\*BH<sup>-</sup> state energies are not known. From a theoretical point of view the question of the  $T$  dependence of the energies of the charge-transfer states is difficult. We simply note the  $Q_y$ -state energies of the accessory B and H molecules are essentially  $T$  independent. In what follows we assume that the  $T$  dependences of the state energies for P\*B<sup>-</sup>H or P\*BH<sup>-</sup> are weak relative to P\*. The dashed theoretical curves in Figure 1 were obtained for a  $\Omega_0 - (S_0\bar{\omega} + S_{0,sp}\omega_{sp})$  mismatch of 460 and 60  $\text{cm}^{-1}$  at room temperature and 4 K, respectively,<sup>27</sup> with the temperature dependence of the mismatch governed by the temperature dependence of P\*'s energy.<sup>18</sup> As can be seen, the fit for *Rps. viridis* is very significantly improved while the allowance for a temperature-dependent energy mismatch is of little consequence for *Rb. sphaeroides*. This is due mainly to the fact that  $\Gamma$  for *Rb. sphaeroides* is significantly larger than that for *Rps. viridis*; cf. Tables I and II. Although differences in the RC structures of *Rps. viridis* and *Rb. sphaeroides* suggest that their electronic matrix element(s) could be significantly different, our calculations show that the near identity of and significant difference between their (short-lived) P\* lifetimes at room and liquid helium temperatures can be understood in terms of linear electron-phonon coupling and structural heterogeneity of the normal type for glasslike media. (The value of  $V = 25 \text{ cm}^{-1}$  required for the fits shown in Figure 1 is essentially identical to the value given in ref 25.) Detailed time domain and hole burning measurements at low temperatures would be able to test these ideas.

We believe that the expressions given here provide insight into the question of dispersive kinetics for primary charge separation arising from structural heterogeneity. At the same time we have emphasized the importance of spectral hole burning data in addressing this question. In summary, we conclude that a distribution of energy gaps will not lead to dispersive kinetics at any temperature. This is based on calculations of  $P^*(t)$  for *Rb. sphaeroides* in which  $2\Gamma$  must be  $600 \text{ cm}^{-1}$  rather than  $240 \text{ cm}^{-1}$  (vide supra) before the dispersion would be detectable in data obtained in the low-temperature limit with typical signal-to-noise levels. We are unaware of any data or physical arguments which would support such a large value of  $2\Gamma$ . In any event, a  $2\Gamma$  value of  $600 \text{ cm}^{-1}$  would not yield measurable dispersive kinetics at biological temperatures. If dispersive kinetics are convincingly demonstrated, they must arise either from gross heterogeneity or from a distribution of  $V$  values whose origin is the same as that

for  $\Gamma_1$  ( $\Gamma$ ). The problem then would be to develop a joint probability distribution function for  $V$  and  $\omega_D - \omega_A$ , the adiabatic energy gap.

**Acknowledgment.** Research at the Ames Laboratory was supported by the Division of Chemical Sciences, Office of Basic Energy Sciences, U.S. Department of Energy. Ames Laboratory is operated for the U.S. Department of Energy by Iowa State University under Contract W-7405-Eng-82. R.J.S. acknowledges the support of the National Science Foundation and Grant CHE-90-007 for the research which led to this paper. We thank Jake Petrich and Feng Gai for their analysis of our calculated decay curves for nonexponentiality and useful discussions. We are indebted to Graham Fleming and Jim Norris for providing us with a preprint of ref 12.

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