Electron Spin Polarization in Sequential Electron Transfer. An Example from Iron-Containing Photosynthetic Bacterial Reaction Center Proteins

Seth W. Snyder,[†] Andrea L. Morris,[‡] Stephen R. Bondeson,[§] James R. Norris, and Marion C. Thurnauer*

Chemistry Division, Argonne National Laboratory
Argonne, Illinois 60439
Department of Chemistry, University of Chicago
Chicago, Illinois 60637

Received October 30, 1992

Characteristic electron spin polarization (ESP) is observed during the initial energy conversion steps of natural and artificial photosynthetic systems when they are monitored by time-resolved electron paramagnetic resonance (EPR).[1-3] Correct interpretation of this ESP is essential for elucidating the details of sequential electron transfer leading to stabilized charge separation in these systems. In purple photosynthetic bacterial reaction centers (rcs), these steps are

$$\mathrm{PHQFe^{2+}} \xrightarrow{h\nu} {}^{\mathrm{S}}\mathrm{PHQFe^{2+}} \xrightarrow{k_i \cong 2 \times 10^{13}\ \mathrm{s^{-1}}} \underset{\mathrm{rp1}}{\mathrm{P^+H^-QFe^{2+}}} \xrightarrow[]{\substack{(T < 50\ \mathrm{K})\\ k_{\mathrm{Q}} = 1 \times 10^{10}\ \mathrm{s^{-1}}}} \underset{\mathrm{rp2}}{\mathrm{P^+H[QFe^{2+}]^-}}{}^{4,5} \quad (1)$$

where P is a special pair of bacteriochlorophylls, H is bacteriopheophytin, and Q is ubiquinone (in the rc of *Rhodobacter sphaeroides*), which interacts with Fe^{2+}. If ubiquinone is replaced with quinones that have different redox potentials, k_Q decreases and the lifetime of P^+H^- increases relative to that in native rcs.[6,7] Then, decay of P^+H^- to the triplet, ^TP, and to the ground state, P, can compete with decay to $P^+H[QFe^{2+}]^-$, and the yield of $P^+H[QFe^{2+}]^-$ is reduced.

Electron spin polarization (ESP) was reported from the transient EPR spectrum of P^+HQ^- in iron-decoupled rcs of *Rb. sphaeroides*.[8] Several models have been proposed to explain the ESP.[8-13] These models have differed in the degree that each radical pair (rp) contributes to the ESP. Initial attempts to model the EPR spectra assigned the ESP to the rp mechanism of chemically-induced dynamic electron polarization (cidep).[14] In this version, interactions on P^+H^- produce the ESP observed on P^+HQ^-, and P^+ and Q^- do not interact magnetically. However, the cidep model failed to satisfactorily account for the ESP.[11,15] Additionally, a small dipolar interaction on P^+Q^- is expected.[16-18] Then, the correlated rp polarization (crpp) mechanism[19,20] was applied to explain the ESP. In this model, the ESP results from rapid formation of a spin-correlated interacting rp (P^+Q^-) directly from ^SP, the photoexcited singlet state of P, with no participation of an interacting precursor rp (P^+H^-). The crpp treatment resulted in better fits to the experimental data. However, it is dependent on the assumption that P^+H^- is too short-lived to develop cidep and contribute to the ESP on P^+Q^-. This assumption is not always valid, particularly in the case of iron-depleted rcs.[21-25] Also, the cidep or crpp models alone are not useful for treating all cases of sequential electron transfer, in general.

An extended model that describes ESP in sequential electron transfer has been developed.[26] This model has been applied specifically to the primary reactions of photosynthetic bacteria.[15,27] Interactions on both rp1 and rp2 are included as well as the kinetics for their formation and depletion. Simulated ESP EPR spectra of P^+HQ^- are dependent on k_Q, i.e., they can be dominated by cidep developed on P^+H^-, crpp from P^+HQ^-, or affected by both, depending on the lifetime of P^+H^-.[15,27-29] Experimental results from iron-depleted and Zn-containing rcs support the necessity of applying this model[30,31] and illustrate how the spectral consequences of isotopic variation (protonated vs deuterated) can also provide information regarding the ESP mechanisms. However, quantitative consistency is difficult in the case of iron-depleted rcs because the lifetime of P^+H^- can vary considerably depending on the rc preparation[21-25] and even on the age of the sample.[32] The ESP observed in iron-containing rcs of *Rb. sphaeroides* provides a specific test on a structurally defined system for a complete model for ESP so that it can be applied to study general cases of ESP accompanying sequential electron transfer. Quinone replacement provides the means to vary k_Q. Thus, ESP can be studied in rcs with P^+H^- lifetimes of ~10 ns[33] to 100–200 ps.[34]

[†] Present address: Abbott Laboratories, Drug Design and Delivery, Abbott Park, IL 60064.

[‡] University of Chicago.

[§] Argonne Summer Faculty Research Participant. Permanent address: Department of Chemistry, University of Wisconsin, Stevens Point, WI 54481.

(1) Hoff, A. J. *Q. Rev. Biophys.* **1984**, *17*, 153–282.

(2) Wasielewski, M. R.; Gaines, G. L., III; O'Neil, M. P.; Svec, W. A.; Niemczyk, M. P. *J. Am. Chem. Soc.* **1990**, *112*, 4559–4560.

(3) Hasharoni, K.; Levanon, H.; Tang, J.; Bowman, M. K.; Norris, J. R.; Gust, D.; Moore, T. A.; Moore, A. L. *J. Am. Chem. Soc.* **1990**, *112*, 6477–6481.

(4) Deisenhofer, J., Norris, J. R., Eds. *The Photosynthetic Reaction Center*; Academic Press: New York, in press.

(5) Kirmaier, C.; Holten, D.; Parson, W. W. *Biochim. Biophys. Acta* **1985**, *810*, 33–48.

(6) Gunner, M. R.; Robertson, D. E.; Dutton, P. L. *J. Phys. Chem.* **1986**, *90*, 3783–3795.

(7) Gunner, M. R.; Dutton, P. L. *J. Am. Chem. Soc.* **1989**, *111*, 3400–3412.

(8) Hoff, A. J.; Gast, P.; Romijn, J. C. *FEBS Lett.* **1977**, *73*, 185–190.

(9) Gast, P. Ph.D. Dissertation, University of Leiden, Leiden, The Netherlands, 1982.

(10) de Groot, A.; Gast, P.; Hoff, A. J. In *Advances in Photosynthesis Research*; Sybesma, C., Ed.; Martinus Nijhoff: Den Hague, 1984; Vol. I, pp 215–218.

(11) Hore, P. J.; Watson, E. T.; Pederson, J. B.; Hoff, A. J. *Biochim. Biophys. Acta* **1986**, *849*, 70–76.

(12) Hore, P. J.; Hunter, D. A.; McKie, C. D.; Hoff, A. J. *Chem. Phys. Lett.* **1987**, *127*, 495–500.

(13) Hore, P. J. In *Advanced EPR, Applications in Biology and Biochemistry*; Hoff, A. J., Ed.; Elsevier: Amsterdam, 1989; pp 405–440.

(14) Adrian, F. J. *Rev. Chem. Int.* **1979**, *3*, 3–43.

(15) Morris, A. L.; Norris, J. R.; Thurnauer, M. C. In *Reaction Centers of Photosynthetic Bacteria*; Michel-Beyerle, M.-E., Ed.; Springer Series in Biophysics, Springer-Verlag: Berlin, 1990; Vol. 6, pp 423–435.

(16) Deisenhofer, J.; Epp, O.; Miki, K.; Huber, R.; Michel, H. *J. Mol. Biol.* **1984**, *180*, 385–398.

(17) Chang, C. H.; Tiede, D. M.; Tang, J.; Smith, U.; Norris, J. R.; Schiffer, M. *FEBS Lett.* **1986**, *205*, 82–86.

(18) Allen, J. P.; Feher, G.; Yeates, T. O.; Rees, D. C.; Deisenhofer, J.; Michel, H.; Huber, R. *Proc. Nat. Acad. Sci. U.S.A.* **1986**, *83*, 8589–8593.

(19) Thurnauer, M. C.; Norris, J. R. *Chem. Phys. Lett.* **1980**, *76*, 557–561.

(20) Closs, G. L.; Forbes, M. D. E.; Norris, J. R. *J. Phys. Chem.* **1987**, *91*, 3592–3599.

(21) Blankenship, R. E.; Parson, W. W. *Biochim. Biophys. Acta* **1979**, *545*, 429–444.

(22) Debus, R. J.; Feher, G.; Okamura, M. Y. *Biochemistry* **1986**, *25*, 2276–2287.

(23) Kirmaier, C.; Holten, D.; Debus, R. J.; Feher, G.; Okamura, M. Y. *Proc. Nat. Acad. Sci. U.S.A.* **1986**, *83*, 6407–6411.

(24) Agalidis, I.; Nuijs, A. M.; Reiss-Husson, F. *Biochim. Biophys. Acta* **1987**, *890*, 242–250.

(25) Liu, B.-L.; van Kan, P. J. M.; Hoff, A. J. *FEBS Lett.* **1991**, *289*, 23–28. Schelvis, J. P. M.; Liu, B.-L.; Aartsma, T. J.; Hoff, A. J. *Biochim. Biophys. Acta* **1992**, *1102*, 229–236.

(26) Norris, J. R.; Morris, A. L.; Thurnauer, M. C.; Tang, J. *J. Chem. Phys.* **1990**, *92*, 4239–4249.

(27) Morris, A. L. Ph.D. Dissertation, The University of Chicago, Chicago, IL, 1992.

(28) Thurnauer, M. C.; Feezel, L. L.; Morris, A. L.; Smith, U.; Norris, J. R. In *Current Research in Photosynthesis*; Baltscheffsky, M., Ed.; Kluwer Academic Publishers: 1989; Vol. 1, pp 181–184.

(29) Snyder, S.; Thurnauer, M. C. In *The Photosynthetic Reaction Center*; Deisenhofer, J., Norris, J. R., Eds.; Academic Press, Inc.: New York, in press.

(30) Feezel, L. L.; Gast, P.; Smith, U. H.; Thurnauer, M. C. *Biochim. Biophys. Acta* **1989**, *974*, 149–155.

(31) Feezel, L. L.; Reiss-Husson, F.; Agalidis, I.; Smith, U. H.; Thurnauer, M. C.; Norris, J. R. *Appl. Magn. Reson.* **1990**, *1*, 255–265.

(32) Netzel, T. L. In *Biological Events Probed by Ultrafast Laser Spectroscopy*; Academic Press, Inc.: New York, 1982; pp 79–117.

0002-7863/93/1515-3774$04.00/0

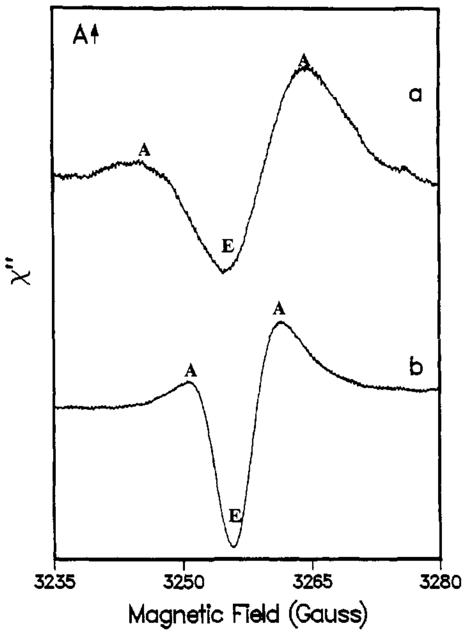

Figure 1. Transient EPR signal of P^+ in the state $P^+H[QFe^{2+}]^-$ from rcs of *Rb. sphaeroides* R26 isolated as described previously.[43] (a) Native iron-containing protonated rcs; (b) 99.7% deuterated rcs (isolated from cells grown in D_2O (99.7%) on deuterated substrates).[44,45] Signals were monitored by direct-detection time resolved EPR techniques as described in ref 46. Signals were collected 2 μs after pulsed laser (10 Hz, 585 nm, 5 mJ/pulse) excitation. The polarization pattern was essentially unchanged for the delay time range examined (600 ns to 5 μs). $T \cong 13$ K.

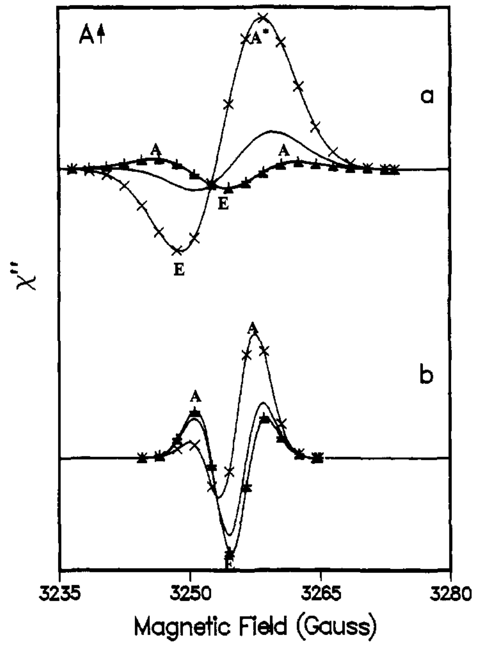

Figure 2. Calculated EPR spectral dependence for P^+ in $P^+H[QFe^{2+}]^-$ on k_Q using extended ESP model for sequential electron transfer[15,27] with the following parameters: dipolar coupling ($P^+H^- = -4.5$ G, $P^+[QFe^{2+}]^- = -1.5$ G); exchange coupling ($P^+H^- = 7$ G, $P^+[QFe^{2+}]^- = 0$ G); g factors ($P^+/g_{xx} = 2.0033$; $g_{yy} = 2.0025$; $g_{zz} = 2.0021$[47]; $H^-/g = 2.0035$; $[QFe^{2+}]^-/g_{xx} = 1.67$; $g_{yy} = 1.67$; $g_{zz} = 1.84$); P^+H^- decay paths (to $P/k_S = 3 \times 10^6\ s^{-1}$; to $^TP/k_T = 4 \times 10^8\ s^{-1}$). (a) Protonated case, Gaussian envelopes: $P^+ = 9.5$ G; $H^- = 13.5$ G. (b) Deuterated case, Gaussian envelopes: $P^+ = 4.5$ G; $H^- = 5.7$ G. The orientations of P^+ magnetic axes are defined relative to the crystal axes by the Euler angles (θ_1, 75°; θ_2, 130°; and θ_3, 175°). The orientation of the ubiquinone was determined by X-ray crystallography,[16-18] and its magnetic axes are described in ref 48. (×) $k_Q = 2 \times 10^8\ s^{-1}$; (—) $k_Q = 8 \times 10^8\ s^{-1}$; (+) $k_Q = 5 \times 10^9\ s^{-1}$; (▲) $k_Q = 1 \times 10^{10}\ s^{-1}$.

We report ESP EPR spectra obtained from native protonated and deuterated (99.7%) iron-containing rcs of *Rb. sphaeroides* R26 and interpret the results together with those from quinone-replaced rcs using the extended ESP model for sequential electron transfer. The time-resolved P^+ EPR signals from $P^+H[QFe^{2+}]^-$ in protonated and deuterated rcs of *Rb. sphaeroides* R26 are presented in Figure 1. Both signals exhibit a polarization pattern of absorption(A)/emission(E)/absorption(A).

The A/E/A pattern for P^+ from $P^+H[QFe^{2+}]^-$ can be understood in the context of the ESP model, which includes the consequences of sequential rp formation. Simulated spectra[35] for P^+ obtained using this model are presented in Figure 2. These simulations demonstrate the dependence of the ESP on k_Q. For the ^{1}H case, when $k_Q \gtrsim 8 \times 10^8\ s^{-1}$, the crpp developed on rp2 dominates yielding A/E/A polarization. As k_Q is decreased, the cidep which develops on rp1 affects the spectrum. When $k_Q \lesssim 2 \times 10^8\ s^{-1}$, the effects of cidep developed by rp1 dominate, yielding E/A* polarization[39] with concomitant g shifts, because significant cancellation of the effects of crpp occur when a rp has a small dipolar coupling relative to the unresolved line widths.[27,29,30] This cancellation is less for the narrower P^+ line width in deuterated rcs, and the smaller deuterium hyperfine coupling decreases the development of cidep by rp1. Thus, the predicted A/E/A pattern persists throughout the range of k_Q values used for Figure 2b. In both the protonated and deuterated cases, intensity changes as a function of k_Q are also dependent upon the P^+ yield. Comparing Figures 1 and 2 shows that the observation of A/E/A ESP for P^+ in $P^+H[QFe^{2+}]^-$ is predicted for the case of native rcs in which k_Q is on the order of $10^{10}\ s^{-1}$. The calculated spectra of Figure 2a predict that as k_Q decreases there is a smooth progression to the E/A* pattern[40] that was observed in several protonated quinone-replaced rcs.[33]

The results from native rcs (Figure 1 and ref 34) together with previous results from quinone-replaced rcs[33] can be understood within the framework of one model for ESP. The extended model for ESP in sequential electron transfer can be used to follow changes in k_Q such as those that occur in genetically modified rc proteins[42] or to follow sequential electron transfer in model photosynthetic systems.[2,3] A detailed quantitative description of the effects shown in Figure 2, including ESP in quinone-replaced reaction centers, will be presented in a subsequent publication.

Acknowledgment. This work was supported by the U.S. Department of Energy, Office of Basic Energy Sciences, Division of Chemical Sciences under contract W-31-109-Eng-38. We thank U. Smith for reaction center preparation, Y. Zhang for technical assistance, and D. Budil for his participation during the initial stages of this project.

---

(33) Gunner, M. R.; Robertson, D. E.; LoBrutto, R. L.; McLaughlin, A. C.; Dutton, P. L. In *Progress in Photosynthesis Research*; Biggins, J., Ed.; Martinus Nijhoff: Dordrecht, 1987; Vol. I, pp 217–220.

(34) Proskuryakov, I. I.; Shkuropatov, A. Ya.; Sarvazian, N. A.; Shuvalov, V. A. *Dokl. Akad. Nauk SSSR (Russian)* **1991**, *320*, 1006–1008.

(35) The $[QFe^{2+}]^-$ complex is modeled by a spin $^1/_2$ system, a reasonable approximation of low temperatures.[36-38]

(36) Butler, W. F.; Johnston, D. C.; Shore, H. B.; Fredkin, D. R.; Okamura, M. Y.; Feher, G. *Biophys. J.* **1980**, *32*, 967–992.

(37) Butler, W. F.; Calvo, R.; Fredkin, D. R.; Isaacson, R. A.; Okamura, M. Y.; Feher, G. *Biophys. J.* **1984**, *45*, 947–973.

(38) Calvo, R.; Butler, W. F.; Isaacson, R. A.; Okamura, M. Y.; Fredkin, D. R.; Feher, G. *Biophys. J.* **1982**, *37*, 111a.

(39) An * indicates enhanced absorption.

(40) The predicted and observed change to E/A* as k_Q decreases confirms[41] that the sign of the exchange interaction on rp1, J_{PH}, is positive.

(41) Hore, P. J.; Hunter, D. A.; Riley, D. J.; Semlyen, J. J.; van Wijk, F. G. H.; Schaafsma, T. J.; Gast, P.; Hoff, A. J. *Res. Chem. Int.* **1991**, *16*, 127–139.

(42) Coleman, W. J.; Youvan, D. C. *Annu. Rev. Biophys. Biophys. Chem.* **1990**, *19*, 333–367.

(43) Wraight, C. A. *Biochim. Biophys. Acta* **1979**, *548*, 309–327.

(44) Crespi, H. L. In *Methods in Enzymology*; Packer, L., Ed.; Academic Press: New York, 1982; Vol. 88, pp 3–5.

(45) Crespi, H. L. In *Stable Isotopes in the Life Sciences*; International Atomic Energy Agency: Vienna, 1977; pp 111–121.

(46) Rustandi, R. R.; Snyder, S. W.; Biggins, J.; Norris, J. R.; Thurnauer, M. C. *Biochim. Biophys. Acta* **1992**, *1101*, 311–320.

(47) Burghaus, O.; Plato, M.; Bumann, D.; Neumann, B.; Lubitz, W.; Möbius, K. *Chem. Phys. Lett.* **1991**, *185*, 381–386.

(48) Hales, B. F. *J. Am. Chem. Soc.* **1975**, *97*, 5993–5997.