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Orientation dependence of the EPR signal from the reduced iron-quinone complex in a single crystal of the reaction center protein from *Rhodopseudomonas viridis*

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In the light-induced charge separation that takes place in the bacterial photosynthetic reaction center protein a quinone molecule (Q) is reduced. The EPR spectrum of this reduced quinone is strongly broadened which is assumed to arise from interaction with the high spin ($S=2$) Fe^{2+}, which is located near Q. Computer simulations of this EPR spectrum have been somewhat limited by the lack of structure in the EPR spectrum. The availability of single crystals of the reaction center protein offers the possibility to analyze the spectrum of the reduced iron-quinone complex (FeQ^-) in much greater detail than ever before. We have therefore measured the EPR spectrum of FeQ^- using single crystals from the reaction center protein from the photosynthetic bacterium *Rhodopseudomonas viridis*. Transitions were obtained in the g value range of 1.7–1.9 for both crystal orientations, i.e. for rotation along and rotation perpendicular to the crystal's long axis. Strong line broadening was observed at many orientations, which could indicate a heterogeneity in the zerofield parameters of Fe^{2+}.

Photosynthesis; Reaction center crystal; EPR; Iron-quinone acceptor

1. INTRODUCTION

The primary charge separation in bacterial photosynthesis takes place in a special membrane-bound protein called the reaction center (RC). This protein contains all the chromophores that are involved in the primary charge separation reaction. Upon light absorption a bacterio-chlorophyll dimer (P) is oxidized and, through the reduction of a bacterio-pheophytin (I), the primary quinone acceptor (Q_A) is reduced; at room temperature the reduction of Q_A is followed by the reduction of Q_B, the secondary quinone acceptor. These quinone molecules are magnetically coupled to an iron atom, which is located between the two quinones. The function of this iron atom is still not well understood. Removal of the iron has a profound effect on the lifetime of the intermediate pair P^+I^- [1], but does not appear to have a large influence on the electron transfer rate from Q_A to Q_B [1]. The presence of the iron atom has its most dramatic effect on the EPR spectrum of the reduced quinone [2,3]. When the iron atom has been chemically removed, the reduced quinone exhibits a narrow EPR spectrum of about 8 G at g = 2.0046; with EPR at 35 GHz a small g anisotropy is observed [4]. When the iron is coupled to the quinone, however, the EPR spectrum is strongly broadened and extends over several thousands of gauss. Several attempts have been made to simulate this spectrum, both in randomly oriented RCs and in partially oriented whole cells [5,6]. In these simulations it is assumed that the broadening of the spectrum is due to existence of an exchange coupling that couples the S = 2 electron spin of Fe^{2+} to the S = 1/2 electron spin of the quinone radical.

The availability of single crystals of this RC pro-

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tein from two types of photosynthetic bacteria, i.e. *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides* R-26 [7–9], offers a unique possibility to verify the validity of the model that has been proposed to explain the EPR spectrum of FeQ^-. Although the crystals have at the most a volume of 3 μl, it has been shown that EPR spectra can be obtained from these crystals [10–14]. This was first done by Gast et al. [10] who measured the angular dependence of the EPR signal of the primary donor triplet state (P^T), which is formed when the primary quinone acceptor is reduced prior to light excitation, in a single crystal from *Rps. viridis*. The detection of this triplet state in the crystals showed that not only are these RCs still highly active after the long crystallization procedure, but also that the crystals can be cooled to 5 K without disturbing the crystal ordering. The same kind of experiments were later repeated with single crystals from *Rb. sphaeroides* wt and the carotenoidless mutant R-26 [11,13,14].

We report here on the detection of the EPR spectrum of FeQ^- in a single crystal from *Rps. viridis*. Only part of the total FeQ^- EPR spectrum is obtained: the signals are only observed for a certain range of crystal rotations and vanish at other orientations because of line broadening. A preliminary attempt was made to simulate these spectra.

2. MATERIALS AND METHODS

Crystals from *Rps. viridis* were grown as in [10]; prior to freezing, the crystals were soaked in a mother liquor solution that contained an additional 1.5 M sucrose. EPR spectra were recorded with a Varian E-9 system equipped with a V-line magnet and magnet controller; the magnetic field was calibrated using the EPR signal of the donor triplet state P^T in *Rps. viridis*. Low temperatures were achieved using an Air Products helium flow cryostat. Mounting and handling of the crystal was as in [10]. An IBM-XT computer was used for data acquisition. Computer simulations of the FeQ^- EPR signal were performed with a simulation program kindly provided by Dr J.J. Villafranca, that was modified for handling the specific Hamiltonian; diagonalization of the Hamiltonian was done with the subroutine EIGCH from the EISPACK package from Argonne National Laboratory [15]. Transition intensities were not included in the calculations.

3. RESULTS

Fig.1 shows the EPR spectra that were obtained from a single crystal from *Rps. viridis* at 4.2 K with 10 mW microwave power. Fig.1a shows the results for rotation of the crystal perpendicular to the magnetic field and along the crystal long axis; fig.1b shows the same for rotation perpendicular to the crystals long axis. Similar spectra were found in several experiments using different crystals. It is seen that the signals are strongly anisotropic, with shifts up to 16 G per degree. The signals vary widely in line width: 30–100 G in fig.1a, 70–200 G in fig.1b. We have investigated whether this line broadening was due to the crystal handling by varying the incubation time in the sucrose/mother liquor solution. No difference was found between a 1-min and a 30-min incubation time. When the redox potential was raised by soaking the crystal in a solution containing ferricyanide, these signals were not observed. This observation and the range of g values for which

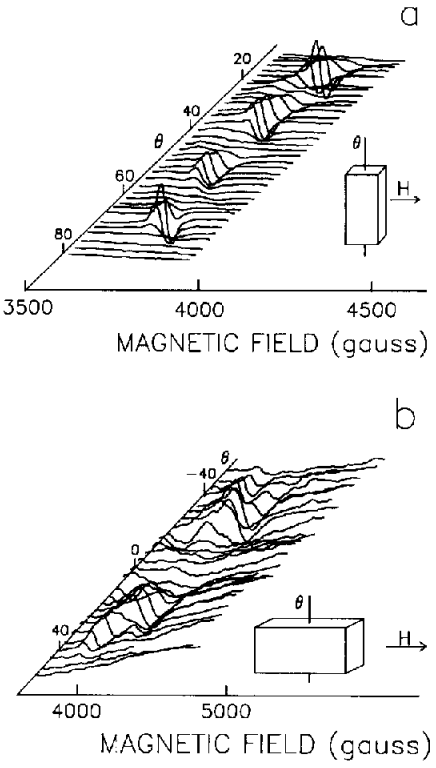

Fig.1. EPR spectra obtained at 4.2 K and 10 mW microwave power from an RC crystal from *Rps. viridis* as a function of rotation (θ) perpendicular to the magnetic field: (a) rotation along the crystal's long axis; (b) rotation perpendicular to the crystal's long axis. The 'zero' of the goniometer was determined using the EPR signal of the donor triplet state.

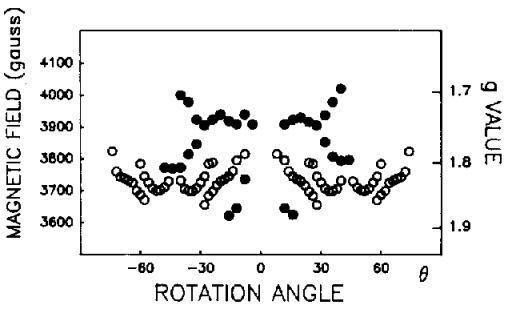

Fig.2. Resonance positions for the two types of rotations: (○) rotation along the crystal's long axis; (●) rotation perpendicular to the crystal's long axis. In the range of negative θ and rotation along the long axis, we measured only a few angles which were found to be the mirror image of the transitions found for positive θ. Therefore the transitions found for positive θ were copied, for clarity.

signals were obtained are indications that these signals arise from the reduced FeQ complex. A search for signals at both lower and much higher magnetic field (1500–13000 G) was performed at 4.2 K, but no other signals could be observed. Also the temperature modulation technique [4,5] did not improve the signal intensity such that other transitions at higher or lower magnetic fields could be observed.

Fig.2 shows the resonance values of the transitions found in fig.1. It is seen that the data have the same symmetry relation as was found for the donor triplet state in the same type of crystal (fig.2 in [10]).

4. DISCUSSION

The amount of new information that is obtainable with EPR measurements on an RC crystal depends on the extent of anisotropy of the EPR spectrum. When the signal shows angular dependency, information will be obtained that is not obtainable from randomly or partially oriented samples. This places a restriction on what can be measured. Since the crystal has at best a volume of 3 μl, the EPR signals may be weak. However, because the crystal is highly ordered, the intensities may be several orders of magnitude larger than in the randomly oriented case. Therefore it will be possible to measure signals that normally cannot be observed with a microliter sample volume. This advantage however will be strongly reduced when the single line transitions are broadened by crystal defects, crystal misalignments or lifetime effects to such an extent that the linewidth becomes larger than the maximum magnetic field modulation amplitude (in most cases 40 G).

This seems to be the case in these measurements (fig.1): only where the angular dependency is small (i.e. at the turning points) are the signals large. Movement of a few degrees away from these points, causes the lines to broaden and become strongly reduced in intensity. This effect could be due to several factors such as crystal defects, inherent to the crystal or induced by the handling, heterogeneity in the protein or to misalignment of the crystal. It seems unlikely that the crystal handling has a dramatic effect on the linewidth, since we did not find any effect on the incubation time in the sucrose solution. However, cooling to 4.2 K could produce small defects in the crystal which may not have shown up in earlier triplet measurements [10,11] where the anisotropy is more than an order of magnitude smaller. It could also be that the proteins inside the crystal are not homogeneous, leading to a variation in the crystal field parameters of Fe^{2+} which largely determine the EPR spectrum. Finally, the method of mounting such a non-rigid crystal may also be the source of the line broadening. For instance, when the crystal is bent over one degree, the linewidth broadening could be several hundreds of gauss, assuming that a 90° rotation moves the transition field from 3000 to 10000 G. This could be the reason why in fig.1a the linewidths are smaller than in fig.1b.

The assignment of these signals to FeQ^- rather than to oxidized cytochrome (oxidized cytochrome should be present since FeQ is photoreduced, resulting into the accumulation of the state cyt^+ FeQ^-) or possible background signals is based on the following findings: (i) the signals are absent when the crystal is treated with ferricyanide, (ii) the signals were best observed at high microwave power, and (iii) the symmetry pattern in fig.2 excludes that the signals arise from background. A more elegant proof for the identification of these signals as arising from FeQ^- would be to monitor its irreversible photo-accumulation (FeQ is irreversibly reduced at low temperature in *Rps. viridis* because cytochrome acts as a fast donor to P^+); however, we were unable to mount the crystal

under complete darkness (and maintain proper mounting) so that FeQ was already photo-reduced before the experiment. This fact also implies that it is not certain whether Q_A or Q_B is photoreduced in the crystals, because the temperature at which Q^- was accumulated has not been established. But, since more than half of Q_B seems to be absent in the crystallized RCs [16] it is likely that most of the observed signals in these crystals are due to Q_A.

We have attempted to simulate the data using the Hamiltonian:

$$H = \beta H \cdot g_{Fe} \cdot S_{Fe} + g_Q H \cdot S_Q + D[S_z^2 - 1/3S(S+1)]_{Fe} + E(S_x^2 - S_y^2)_{Fe} + JS_{Fe} \cdot S_Q$$

in which D and E are the zero-field splitting parameter of Fe^{2+}, g_{Fe} is its anisotropic g tensor, J is the isotropic exchange interaction between Fe^{2+} and Q^- and g_Q is the isotropic g value of Q^-. The simulation program used three Euler angles to alter the position of the protein with respect to the crystal axes; a fourth angle was used to simulate the rotation of the crystal in the magnetic field.

With this Hamiltonian, five energy levels of Fe^{2+} ($S = 2$) are found. Each level is split due to the interaction with the quinone ($S = 1/2$), resulting in ten energy levels. If we adopt the notion that the zero-field splittings are so large that only the two lowest levels of the Fe^{2+} system are populated at low temperature and that the energy difference between these two levels is much larger than 9 GHz [5] we obtain two transitions per site per orientation. In the crystal space group of *Rps. viridis* there are four magnetically non-equivalent sites; therefore one obtains 8 transitions per angle.

We have carried out a preliminary computer simulation of the data. The values tested for D, E, J, g_{Fe} and g_Q were in the range of those found in [5,6]. Due to the limited range of g values over which we obtained data, it was not possible to select a unique fit. A complete fit of the data has to wait until more datapoints over a larger range of field values are available. The largest improvement would be if the linewidths of the transitions could be reduced, possibly by a more precise procedure for mounting the crystal. If the line broadening is due to variation in the crystal field parameters then EPR at lower frequency may reduce the broadening. Also, measurements at temperatures below 4.2 K may reveal more of the spectrum [5]. In crystals from *Rb. sphaeroides* R-26 electron transfer from FeQ^- to P^+ occurs rapidly even at low temperatures. It therefore could be that the EPR spectrum of FeQ^- in these crystals could be observed better since light modulation can be used. However, the volume of these crystals is almost an order of magnitude smaller.

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REFERENCES

[1] Debus, R.J., Feher, G. and Okamura, M.Y. (1986) Biochemistry 25, 2276–2287.
[2] Okamura, M.Y., Isaacson, R. and Feher, G. (1975) Proc. Natl. Acad. Sci. USA 72, 3491–3495.
[3] Wraight, C.A. (1977) Biochim. Biophys. Acta 459, 525–531.
[4] Feher, G., Okamura, M.Y. and McElroy, J.D. (1972) Biochim. Biophys. Acta 262, 222–226.
[5] Butler, W.F., Calvo, R., Fredkin, D.R., Isaacson, R.A., Okamura, M.Y. and Feher, G. (1984) Biophys. J. 45, 947–973.
[6] Dismukes, G.C., Frank, H.A., Friesner, R. and Sauer, K. (1984) Biochim. Biophys. Acta 764, 253–271.
[7] Michel, H. (1982) J. Mol. Biol. 158, 562–567.
[8] Chang, C.-H., Tiede, D.M., Tang, J., Norris, J.R. and Schiffer, M. (1986) FEBS Lett. 205, 82–86.
[9] Allen, J.P., Feher, G., Yeates, T.O., Rees, D.C., Deisenhofer, J., Michel, H. and Huber, R. (1986) Proc. Natl. Acad. Sci. USA 83, 8589–8593.
[10] Gast, P., Wasielewski, M.R., Schiffer, M. and Norris, J.R. (1983) Nature 305, 451–452.
[11] Gast, P. and Norris, J.R. (1984) FEBS Lett. 179, 326–328.
[12] Allen, J.P. and Feher, G. (1984) Proc. Natl. Acad. Sci. USA 81, 4795–4799.
[13] Frank, H.A., Taremi, S.S., Budil, D.E., Gast, P. and Norris, J.R. (1987) Biophys. J. Abstr. M-PM-F6.
[14] Budil, D.E., Taremi, S.S., Gast, P., Norris, J.R. and Frank, H.A. (1988) J. Isr. Chem., submitted.
[15] Smith, B.T., Boyle, J.M., Dongarra, J.J., Garbow, B.S., Ikebe, Y., Klema, V.C. and Moler, C.B., in: Matrix Eigensystem Routines – Eispack Guide, Springer, New York.
[16] Gast, P., Michalski, T.J., Hunt, J.E. and Norris, J.R. (1985) FEBS Lett. 179, 325–328.