INVESTIGATION OF THE STRUCTURE OF THE REACTION CENTER IN PHOTOSYNTHETIC BACTERIA BY OPTICAL DETECTION OF TRIPLET STATE MAGNETIC RESONANCE

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Summary. Measurements of the triplet state zero-field splitting and intersystem crossing rate constants for isolated bacteriochlorophyll a and for chemically reduced photosynthetic bacteria are utilized to investigate the geometry of the bacteriochlorophyll dimer in the reaction center.

Introduction

We have investigated the triplet state properties of photosynthetic pigments by optical detection of magnetic resonance, with the aim of utilizing these properties as a probe into the makeup of photosynthetic systems (1)(2). The present communication reports on the triplet states of bacteriochlorophyll-containing purple photosynthetic bacteria which have been shown to produce strong triplet state EPR signals upon chemical reduction (3)(4).

Recent work, using both optical spectroscopy and magnetic resonance, has shown that the reaction center in photosynthetic bacteria contains a pair of strongly interacting bacteriochlorophyll molecules which are involved in the initial photoprocesses of the cell (5)(6). Earlier triplet EPR work on reaction centers have shown that the triplet state zero-field splitting is reduced in bacteria compared to the isolated bacteriochlorophyll triplet, due to the delocalization of the triplet excitation over the bacteriochlorophyll dimer (3)(4). Thus, in the triplet state of the bacteriochlorophyll dimer there are appreciable intermolecular interactions resulting in delocalization of the triplet electrons over the bacteriochlorophyll pair and a correspondingly smaller zero-field splitting. Models for the geometry of such a reaction center dimer have been proposed by several groups (5)(7)(8). Since the triplet zero-field splitting and the rates of intersystem crossing of the individual spin sublevels of the dimer triplet state are both functions of the dimer geometry, we utilize the measured zero-field splitting and intersystem crossing rates for bacteriochlorophyll and for the chemically reduced purple bacteria to investigate the geometry of the bacteriochlorophyll pair in the reaction center.

Results and Discussion

Work on triplet excitons in molecular crystals (9) has provided a theoretical description for the triplet states of aggregated organic molecules which we can adapt to the description of the bacteriochlorophyll dimer triplet state. Sternlicht and McConnell have shown (10) that when identical interacting molecules are excited into a triplet state in which the interactions between molecules are larger than the zero-field splitting, the spin hamiltonian describing the system is simply the average of the spin hamiltonians of the isolated molecules. This exciton description can be shown (11)(12) to lead to the following expressions for the eigenfunctions of a triplet dimer in terms of the complete (space and spin) triplet eigenfunctions for the monomer:

$$\Psi^{f}_{x^*}(\pm) = 1/\sqrt{2}[\phi^{f}_{A}\phi^{\circ}_{B}(l_{xA}t_{xA}+l_{yA}t_{yA}+l_{zA}t_{zA})\pm\phi^{\circ}_{A}\phi^{f}_{B}(l_{xB}t_{xB}+l_{yB}t_{yB}+l_{zB}t_{zB})] \quad (1)$$

$$\Psi^{f}_{y^*}(\pm) = 1/\sqrt{2}[\phi^{f}_{A}\phi^{\circ}_{B}(m_{xA}t_{xA}+m_{yA}t_{yA}+m_{zA}t_{zA})\pm\phi^{\circ}_{A}\phi^{f}_{B}(m_{xB}t_{xB}+m_{yB}t_{yB}+m_{zB}t_{zB})] \quad (2)$$

$$\Psi^{f}_{z^*}(\pm) = 1/\sqrt{2}[\phi^{f}_{A}\phi^{\circ}_{B}(n_{xA}t_{xA}+n_{yA}t_{yA}+n_{zA}t_{zA})\pm\phi^{\circ}_{A}\phi^{f}_{B}(n_{xB}t_{xB}+n_{yB}t_{yB}+n_{zB}t_{zB})] \quad (3)$$

where the subscripts A,B refer to the two molecules, ϕ^{f}_{A}, ϕ^{f}_{B} correspond to the monomer triplet spatial wavefunctions, ϕ°_{A}, ϕ°_{B} correspond to the complete monomer ground state wavefunctions, and the t_{iA}, t_{iB} (i = x,y,z) are the zero-field triplet spin functions for the monomers A and B. The l_{iA}, l_{iB}, m_{iA}, m_{iB}, n_{iA}, n_{iB} are the direction cosines of the angles between the principal magnetic axes (x,y,z) of the monomer triplet states and the "average" principal magnetic axes (x^*,y^*,z^*) of the dimer. The triplet dimer wavefunctions group into two sets corresponding to the plus and minus triplet dimer levels, and the properties of these levels can be determined from the triplet properties of the monomer modified by geometrical terms. The expressions 1-3 can be used to calculate the zero-field energies of the dimer (for both the plus and minus levels):

$$X^* = l_x^2X + l_y^2Y + l_z^2Z \qquad (4)$$

$$Y^* = m_x^2X + m_y^2Y + m_z^2Z \qquad (5)$$

$$Z^* = n_x^2X + n_y^2Y + n_z^2Z \qquad (6)$$

where X,Y,Z are the zero-field energies of the monomer and $l_i^2=l_{iA}^2=l_{iB}^2$, $m_i^2=m_{iA}^2=m_{iB}^2$, $n_i^2=n_{iA}^2=n_{iB}^2$. The intersystem crossing rates from the triplet dimer spin sublevels to the ground state may also be written from eq. 1-3 in terms of the dimer geometry and the monomer intersystem crossing rates, k_i, as (12)

$$k_x^*(\pm) = \frac{1}{2}\left|(l_{xA}\pm l_{xB})k_x^{1/2} + (l_{yA}\pm l_{yB})k_y^{1/2} + (l_{zA}\pm l_{zB})k_z^{1/2}\right|^2 \qquad (7)$$

$$k_y^*(\pm) = \frac{1}{2}\left|(m_{xA}\pm m_{xB})k_x^{1/2} + (m_{yA}\pm m_{yB})k_y^{1/2} + (m_{zA}\pm m_{zB})k_z^{1/2}\right|^2 \qquad (8)$$

$$k_z^*(\pm) = \frac{1}{2}\left|(n_{xA}\pm n_{xB})k_x^{1/2} + (n_{yA}\pm n_{yB})k_y^{1/2} + (n_{zA}\pm n_{zB})k_z^{1/2}\right|^2 \qquad (9)$$

Thus, a measurement of the monomer and dimer triplet zero-field splittings and spin sublevel intersystem crossing rates can be utilized to determine features of the dimer geometry.

Table 1 provides the triplet state zero-field splittings and intersystem crossing rate constant for bacteriochlorophyll and for several chemically

Table 1. Triplet state zero-field splittings (cm^{-1}) and depopulation rate constants (sec^{-1}) measured by optical detection of magnetic resonance at 2°K (errors in parentheses).

system	$\|D\|$	$\|E\|$	k_x	k_y	k_z
bacteriochlorophyll a	0.0238	0.0069	2287	3321	661
(in THF)	(0.0005)	(0.0003)	(280)	(572)	(74)
R. Spheroides	0.0188	0.0032	2675	3033	1600
(strain 2.4.1)	(0.0002)	(0.0001)	(300)	(200)	(180)
R. Spheroides	0.0187	0.0031	2660	3183	1595
(R-26 mutant)	(0.0002)	(0.0001)	(130)	(246)	(75)
R. Rubrum	0.0189	0.0033	2105	2885	1335
	(0.0003)	(0.0002)	(182)	(400)	(58)

reduced photosynthetic bacteria, measured by zero-field optical detection of magnetic resonance (1). From these data and eq. 4-9 it is clear that a symmetrical dimer (7) or any arrangement which orients the principal magnetic axes of both molecules in the dimer parallel (8) is not consistent with the observed triplet properties of the bacterial reaction centers. A symmetrical dimer (with a two-fold axis of rotation) would display two inactive spin sublevels in intersystem crossing from the plus state ($k_i(+) = 0$) and one inactive intersystem crossing level in the minus state from eq. 7-9; Table 1 shows all the rate constants in the bacteria to be comparable or larger than those observed in bacteriochlorophyll. The reduction in zero-field splitting relative to bacteriochlorophyll excludes the possibility of a plane-parallel dimer from eq. 4-6. Further, any dimer with both molecules positioned to have all molecular axes parallel would have identical zero-field splitting to bacteriochlorophyll and would display intersystem crossing rates twice those of the monomer in the plus state and zero in the minus state, again inconsistent with the observed results.

The reduction of the triplet zero-field splitting in bacteria compared to bacteriochlorophyll indicates that the molecules are rotated away from plane-parallel; the similarity of k_x^*, k_y^* to k_x,k_y indicates substantial rotation of the in-plane axes of one molecule relative to the other in the dimer and that the intersystem crossing originates from the spin sublevels associated with the plus dimer state. By calculating the range of geometries consistent with the bacteria zero-field splitting from eq. 4-6 and then by using these geometries to calculate the dimer intersystem crossing rates from eq. 7-9, we find that the best fit consistent with all the data is obtained when the normal to the planes of the two molecules make an angle of 48° and the in-plane axes of the two molecules are rotated by approximately 78°. The combination of zero-field splittings and intersystem crossing data appears to produce a unique arrangement for the dimer, up to the limits of error in our experiments. Thus, it would seem that the best model of the bacteriochlorophyll pair in the reaction center of photosynthetic bacteria is a modification of that proposed by Katz and Norris (5), in which the water-linked bacteriochlorophyll units are tilted and rotated to fit the orientational arrangement described above.

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