STRUCTURAL ASPECTS OF THE REACTION CENTER OF PHOTOSYNTHETIC BACTERIA CALCULATED FROM TRIPLET STATE ZERO-FIELD SPLITTINGS

Richard H. CLARKE and David R. HOBART

Department of Chemistry, Boston University, Boston, MA 02215, USA

Received 18 July 1977

1. Introduction

Since the original observation of photoexcited triplet-state EPR spectra in reaction centers of photosynthetic bacteria by Leigh and Dutton [1–4], there has been a considerable amount of magnetic resonance data accumulated on the triplet states of the pigment molecules, reaction centers and whole cell systems of photosynthetic bacteria [5–9]. Zero-field splittings have been measured for the photoexcited triplet state of bacteriochlorophyll *a* and bacteriochlorophyll *b* and for a variety of bacterial systems containing these molecules [7,9]. The triplet state data for the bacterial systems have been interpreted as arising from a bacteriochlorophyll dimer within the reaction center observed when the primary electron acceptor has been reduced [4,6]. It has been shown in previous work [6,8,10], that the triplet state properties of a dimer in which the triplet excitation is shared between the two molecules may be calculated from the properties of the monomer and the geometry of the pair. Therefore, since the bacteriochlorophyll triplet state properties are known, the photoexcited triplet state can be used as a probe to investigate the orientation of the pigment molecules in the special pair [6] present in the reaction center of bacterial systems.

Since the zero-field splittings are available for a large number of bacteria, we have utilized these data, along with the most recently published bacteriochlorophyll pigment zero-field splittings [8,9] to determine the relative orientation of the pair molecules in the reaction center for the presently known photosynthetic bacteria. As has been shown previously [8,10], the zero-field splitting alone does not fix completely the orientation of the dimer molecules relative to one another, but does allow a calculation of the angle of the plane of the ring system of one molecule relative to the other [8,11]. The results of these calculations are presented and compared with triplet-state magnetic resonance measurements of the zero-field splitting of an *in vitro* chlorophyll system investigated by Fong and Koester, a system which is postulated to contain a solvent-linked chlorophyll dimer [12].

2. Methods and materials

A methylcyclohexane:*n*-pentane (1:1) solution containing 10^{-5} M chlorophyll *a* (Sigma Chemical Co.) and 10^{-2} M water was prepared as described by Fong and Koester [12]. The absorbance and low temperature fluorescence of the solution agreed well with that previously reported [7]. Samples of the solution were frozen in quartz tubes for the magnetic resonance experiments.

The zero-field splittings reported in this paper for the chlorophyll solution were measured at 2°K by optically-detected zero-field magnetic resonance (ODMR) spectroscopy, monitoring microwave-induced changes in the fluorescence. The excitation source was the 457.9 nm line of a Spectra-Physics model 164 argon-ion laser. Details of the ODMR method and experimental setup have been published previously [13].

3. Results and discussion

Using the equations of ref. [8], we have calculated the angle between the planes for the reaction center dimers from the reported zero-field splittings in a series of photosynthetic bacteria [7]. Each calculation produced a range of angles, all consistent with the monomer pigment zero-field splitting, the zero-field splitting for the bacterium and the standard deviation reported for the zero-field splitting measurements. The calculations on bacteria containing bacteriochlorophyll *a* were performed using the zero-field splitting values obtained in THF by Clarke et al. [8]; similar results (about 10% smaller) are calculated using the bacteriochlorophyll *a* values of Thurnauer and Norris obtained in a pyridine–toluene solution [9]. The bacteriochlorophyll *b* zero-field splittings are taken from the most recent work of Thurnauer and Norris [9]. Results of all these calculations are presented in table 1.

The most striking feature of the table is the uniformity of angles calculated among the bacteria. All give angles around 50° (± 10°), even with different pigment molecules, as in the case of *Rhodopseudomonas viridis* which contains bacteriochlorophyll *b*. If the triplet state measurements do arise from the special pair within the reaction center, there appears to be a general preferred orientation of the planes of the molecules making up the pair for all the various bacterial systems in which the pigment molecules are tipped relative to one another by about 50°. Of course, the calculations give no indication of the degree of difference in relative alignment of the in-plane axes of the two molecules, but the overall

Table 1
Calculated angle between the molecular planes in the reaction center bacteriochlorophyll dimer of photosynthetic bacteria from the triplet state zero-field splitting

Bacterium[a]		Calculated angle (degrees)
Rhodopseudomonas viridis		
strain NHTC 133	cells	51 ± 9
	chromatophores	50 ± 10
Candida vinosum		
strain D	cells	51 ± 9
	chromatophores	51 ± 9
Rhodopseudomonas sphaeroides		
strain 2.4.1	cells	48 ± 8
strain Ga	cells	46 ± 10
	chromatophores	48 ± 8
strain R-26	cells	46 ± 10
	chromatophores	46 ± 10
	reaction centers	48 ± 10
Rhodopseudomonas capsulata		
strain St. Louis	cells	48 ± 8
strain SB 25	cells	48 ± 8
strain BY 761	cells	48 ± 8
Rhodopseudomonas gelatinosa		
strain I	cells	48 ± 8
Rhodopseudomonas palustris		
strain 2.1.6	cells	50 ± 6
Rhodospillum rubrum		
strain S 1	cells	46 ± 10
strain G9	cells	48 ± 8

[a]Zero-field splitting data for bacteria taken from [7]

orientation of the planes appears roughly constant in all systems.

For comparison we have measured the zero-field splittings in a hydrocarbon solution (a 1:1 mixture of methylcyclohexane and *n*-pentane) containing chlorophyll and an excess of water, a solution proposed as an in vitro model system for the reaction center pair by Fong and Koester [12]. This solution exhibits an absorbance band at 700 nm (A-700 in the Fong-Koester notation), which they conclude is due to a water-linked dimer of chlorophyll *a*. The solution also exhibits fluorescence bands at 680 nm and at 720 nm (L-720 in their notation) at low temperatures [12]. The L-720 peak is also identified with the water-linked chlorophyll dimer; the fluorescence band at 680 nm is most likely the fully ligated monomer [14]. These spectral assignments are supported by the recent work of Boxer and Closs, who synthesized a covalently-linked dimeric derivative of pyrochlorophyllide *a* which can be folded over into a parallel dimer structure in hydrocarbon solvents by the addition of water [15]. In the presence of water the folded pyrochlorophyllide dimer exhibits an absorbance at 700 nm and a long-wavelength fluorescence at 720 nm; in its unfolded form the system fluoresces at 680 nm [15]. We have used ODMR spectroscopy [8,13] to measure the zero-field splittings in the chlorophyll–water in hydrocarbon solution, detecting the zero-field magnetic resonance transitions on the 680 nm peak (monomer) and the 720 nm peak (dimer). The transitions detected at 680 nm occur at 728 MHz and 947 MHz; the transitions detected at 720 nm are found at 714 MHz and 923 MHz. The similarity of the two sets of values immediately suggests that the dimeric species must be very close to a plane-parallel (or plane-antiparallel) configuration. We calculate that the planes of the two molecules in the dimer present in the solution are within 10 to 14 degrees of being parallel.*

*ODMR measurements have also been done in our lab on the Boxer-Closs dimer in its folded and unfolded forms. The frequencies of the ODMR transitions in the two forms are very close to those obtained at 680 nm and 720 nm for the solution described above, again implying a geometry in the folded form in which the ring systems are in a parallel (or antiparallel) configuration.

The contrast between the results of the calculations for the bacterial systems and the in vitro water-linked chlorophyll dimer is perhaps not unexpected. In the presence of water chlorophyll locks into a dimeric structure, as predicted by Fong [16] and by Katz et al. [17], whose orientation is determined by the solvent coordinating to the metal center of one chlorophyll molecule and hydrogen bonding to a second [16,17]. The calculated geometry is very close to the predicted parallel orientation; the small angle of rotation away from exact alignment calculated above may be due to the approximations inherent in the simple exciton calculation, rather than a real geometrical feature of the dimer. In the case of bacterial systems, however, the calculations leave little doubt that the dimer is non-planar. In these systems the dimer orientation will be determined not only by interactions linking the pair, but also by the environmental interactions from surrounding proteins and other nearby pigment molecules, all of which must be considered in determining the most stable configuration of the special pair in the reaction center in vivo.

Acknowledgements

Supported in part by the US Energy Research and Development Administration under contract No. E(11-1)-2570, and by the Petroleum Research Fund, administered by the American Chemical Society.

References

[1] Dutton, P. L., Leigh, J. S. and Siebert, M. (1972) Biochem. Biophys. Res. Commun. 46, 406–413.
[2] Dutton, P. L., Leigh, J. S. and Reed, D. W. (1973) Biochim. Biophys. Acta 292, 654–664.
[3] Wraight, C. A., Leigh, J. S., Dutton, P. L. and Clayton, R. K. (1974) Biochim. Biophys. Acta 333, 401–408.
[4] Leigh, J. S. and Dutton, P. L. (1974) Biochim. Biophys. Acta 357, 67–77.
[5] Uphaus, R. A., Norris, J. R. and Katz, J. J. (1974) Biochim. Biophys. Res. Commun. 61, 1057–1063.
[6] Thurnauer, M. C., Katz, J. J. and Norris, J. R. (1975) Proc. Natl. Acad. Sci. USA 72, 3270–3274.

[7] Prince, R. C., Leigh, J. S. and Dutton, P. L. (1976) Biochim. Biophys. Acta 440, 622–636.
[8] Clarke, R. H., Connors, R. E., Frank, H. A. and Hoch, J. C. (1977) Chem. Phys. Lett. 45, 523–528.
[9] Thurnauer, M. C. and Norris, J. R. (1977) Chem. Phys. Lett. 47, 100–105.
[10] Ckarke, R. H., Connors, R. E. and Frank, H. A. (1976) Biochem. Biophys. Res. Commun. 71, 671–675.
[11] Hagele, W. (1976) paper, 3rd Int. Sem. Energy Transfer in Condensed Matter, Prague, Czech.
[12] Fong, F. K. and Koester, V. J. (1976) Biochim. Biophys. Acta 423, 52–64.
[13] Clarke, R. H. and Hofeldt, R. H. (1975) J. Chem. Phys. 61, 4582–4587.
[14] Kleibeuker, J. F., Van der Bent, S. J. and Schaafsma, T. J. (1976) paper, 3rd Int. Sem. Energy Transfer in Condensed Matter, Prague, Czech.
[15] Boxer, S. G. and Closs, G. L. (1976) J. Am. Chem. Soc. 98, 5406–5408.
[16] Fong, F. K. (1975) Appl. Phys. 6, 151–166.
[17] Shipman, L. L., Cotton, T. M., Norris, J. R. and Katz, J. J. (1976) Proc. Natl. Acad. Sci. USA 73, 1791–1794.