Triplet-State Electron Spin Resonance of Chlorophyll a and b Molecules and Complexes in PMMA and MTHF

II: Interpretation of the Experimental Results

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Z. Naturforsch. **33a**, 94—97 (1978); received November 4, 1977

ESR spectra of various triplet species, obtained with solutions of chlorophyll a or chlorophyll b in PMMA or MTHF, are attributed to isolated chlorophyll molecules, to chlorophyll-solvent complexes and to dimeric structures. The structure of the proposed dimer is determined using the experimental results for the fine-structure and the rate constants and by assuming an incoherent triplet-energy exchange. Based on this model the structure of the reaction center in photosynthetic bacteria was also reevaluated using the experimental data which were published by various authors.

1. Introduction

In the preceding paper [1] we have presented experimentally determined fine-structure and rate constants for chlorophyll a (chl a) and chlorophyll b (chl b) in polymethylmethacrylate (PMMA) or methyltetrahydrofurane (MTHF) as a function of the chlorophyll concentration. In this paper we attempt to discuss these results and to assign the various sets of parameters to certain microscopic chlorophyll structures. In Section 2 we will discuss the low- and medium-concentration fine-structure data (A0, A1, A2 and B0, B1, B2, B3 of Reference [1]. The high concentration systems (B4 and B5) appear to originate from special chlorophyll dimers. Therefore in Section 3 we will briefly outline the procedure to estimate such dimer data from the data of the participating monomers. As an example we will apply this procedure to the ESR data on bacterio-chlorophyll in vivo obtained by various authors [2—8]. We will briefly compare the proposed method to the previous evaluation presented by Clarke and coworkers [7, 8]. Finally in Section 4 we will attempt to derive models for dimeric structures, B4 and B5. The results are in fair agreement with those obtained by Houssier and Sauer [9, 10] who based their analysis on the optical properties of chlorophyll and protochlorophyll pigments.

2. Fine-structure Constants at Low and Medium Concentrations

The observed fine-structure constants of chl a and chl b in PMMA and MTHF are compiled in Table 1 of Reference [1]. A comparison of these numbers shows that the fine-structure constants of chl a and of chl b are different in different matrices. This observation is in agreement with previous results of other authors concerning chl a and chl b in various matrices [11—15]. Comparing all these results, it is noteworthy that the value of D for both chl a and chl b in PMMA has its maximum value observed in any of these matrices, whereas in MTHF the low concentration value of D is 5 or 8% lower than that in PMMA for chl a or chl b, respectively.

PMMA acts essentially as a nonpolar solvent and it is therefore reasonable that chlorophyll does not form complexes with the solvent molecules because of its polymeric structure. Therefore it is likely that A0 and B0 are the monomeric forms of isolated chl a and chl b molecules. On the other hand MTHF is a polar solvent which is likely to form chlorophyll-solvent complexes, since the magnesium atom in chlorophyll has two nonsaturated coordinations. Two types of such complexes are feasible: chlorophyllmonosolvate (chlorophyll-ligand: chl-L) and chlorophylldisolvate (ligant-chlorophyll-ligand: L-chl-L). Since this coordination envolves the "negative" oxygen end of the MTHF molecule, the Coulomb attraction force for the two unpaired electrons in the chlorophyll triplet state is lowered thereby increasing their average distance and consequently decreasing their dipolar interaction.

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Thus D should increase, when proceeding from L-chl-L to chl-L and to chl. This is exactly what is observed with chl b in MTHF. Thus we may tentatively assign B1 to the chlorophylldisolvate, B2 to the chlorophyllmonosolvate and B3 to the isolated chl b. According to the model B2 and B3 should be formed only, if the concentration is too high to allow a complete solvation of the chlorophylls. Therefore, in an effort to obtain a very crude estimate of the critical concentration above which B2 and B3 are expected to occur, let us assume that it is necessary that the probability for two nearest neighbouring chlorophyll molecules to be closer than a chlorophyll diameter is at least 10%, in order to yield B2 and B3 in a detectable concentration. Based on this assumption we can estimate the critical chlorophyll concentration to be in the order of 5×10^{-3} mole/l, a concentration which is in rough agreement with the experimentally observed critical concentration. It is therefore reasonable to tentatively propose the assignment given in Table 1.

Table 1. Fine-structure constants of various chlorophyll solvates in MTHF.

Triplet-system	Fine-structure constants [10^{-4} cm^{-1}]		Assignment
	D	E	
A1	288 ± 3	42 ± 3	MTHF · Chl a · MTHF
A2	291 ± 3	59 ± 3	MTHF · Chl a
B1	294 ± 3	49 ± 3	MTHF · Chl b · MTHF
B2	315 ± 3	87 ± 3	MTHF · Chl b
B3	325 ± 3	29 ± 5	Chl b

Two limitations of this assignment should be noted:

i) it is not possible to even qualitatively predict the changes in E;

ii) it is unknown why the isolated chl a is not observed as A3.

Unexplained in this scheme are furthermore the high-concentration species B4 and B5. It is tempting to assign them to dimers of either of the species B1, B2 or B3. In the following section we will therefore briefly outline how the fine-structure and the rate constants of a dimer in a given configuration can be estimated from the monomer data.

3. Determination of the Configuration of Dimers Using Fine-Structure and Kinetic Constants

It is well known that the triplet state properties of a dimer, in which the excitation energy is shared between a pair of molecules, can be calculated in a straightforward way from those of the monomer and the geometry of the pair [16—18]. Such triplet pair properties have been studied to a large extend in organic molecular crystals. In most of these pairs the intermolecular interaction was found to be larger than the triplet zero field splitting. It seems reasonable that this strong coupling case applies for chlorophyll dimers, too, and we will restrict our discussion to this case. Furthermore we must distinguish whether the triplet energy is changed in a coherent or an incoherent way. In the former case the proper pair eigenstates are the symmetric or antisymmetric linear combinations of the monomer states [19] and must be used to calculate the expectation values of the physical quantities. This procedure has been used by Clarke and coworkers [7, 8]. However, in this case it is difficult, for instance in calculating the rate constants for the depopulation, to take into account the transitions from the triplet dimer levels into vibronic levels of the singlet ground state and we have not been able to arrive at an unambiguous solution. On the other hand, in organic molecular crystals evidence for a coherent exchange of triplet energy is sparse and has been obtained only at very low temperatures [20—22]. We have therefore chosen the latter model of an incoherent exchange in our attempt to estimate the dimer geometry by comparing its zero-field splitting and rate constants to the corresponding monomer data. In this model the dimer fine-structure eigenvalues and transition probabilities are given by a weighted average of the corresponding monomer quantities.

It is straightforward in this model to derive the following expressions for the dimer fine-structure-tensor eigenvalues, X^{AB}, Y^{AB}, and Z^{AB} and for the dimer rate constants for the triplet-level depopulation, ${k_x}^{AB}$, ${k_y}^{AB}$ and ${k_z}^{AB}$ [23]:

$$\begin{aligned} 2X^{AB} = {} & [({\alpha_x}^A)^2 + ({\alpha_x}^B)^2]\,X + [({\beta_x}^A)^2 + ({\beta_x}^B)^2]\,Y \\ & + [({\gamma_x}^A)^2 + ({\gamma_x}^B)^2]\,Z\,, \end{aligned} \tag{1a}$$

$$\begin{aligned} 2Y^{AB} = {} & [({\alpha_y}^A)^2 + ({\alpha_y}^B)^2]\,X \\ & + [({\beta_y}^A)^2 + ({\beta_y}^B)^2]\,Y \\ & + [({\gamma_y}^A)^2 + ({\gamma_y}^B)^2]\,Z\,, \end{aligned} \tag{1b}$$

$$\begin{aligned} 2Z^{AB} &= [(\alpha_z{}^A)^2 + (\alpha_z{}^B)^2]\, X \\ &\quad + [(\beta_z{}^A)^2 + (\beta_z{}^B)^2]\, Y \\ &\quad + [(\gamma_z{}^A)^2 + (\gamma_z{}^B)^2]\, Z\,, \end{aligned} \tag{1c}$$

$$\begin{aligned} 2k_x{}^{AB} &= [(\alpha_x{}^A)^2 + (\alpha_x{}^B)^2]\, k_x \\ &\quad + [(\beta_x{}^A)^2 + (\beta_x{}^B)^2]\, k_y \\ &\quad + [(\gamma_x{}^A)^2 + (\gamma_x{}^B)^2]\, k_z\,, \end{aligned} \tag{2a}$$

$$\begin{aligned} 2k_y{}^{AB} &= [(\alpha_y{}^A)^2 + (\alpha_y{}^B)^2]\, k_x \\ &\quad + [(\beta_y{}^A)^2 + (\beta_y{}^B)^2]\, k_y \\ &\quad + [(\gamma_y{}^A)^2 + (\gamma_y{}^B)^2]\, k_z\,, \end{aligned} \tag{2b}$$

$$\begin{aligned} 2k_z{}^{AB} &= [(\alpha_z{}^A)^2 + (\alpha_z{}^B)^2]\, k_x \\ &\quad + [(\beta_z{}^A)^2 + (\beta_z{}^B)^2]\, k_y \\ &\quad + [(\gamma_z{}^A)^2 + (\gamma_z{}^B)^2]\, k_z\,. \end{aligned} \tag{2c}$$

In these expressions the quantities $\alpha_x{}^A$ etc. are the direction cosines of the angles between the principal magnetic axes of the monomer A or B ($\hat{x}^A$, $\hat{y}^A$, $\hat{z}^A$) and the "averaged" principal magnetic axes of the dimer ($\hat{x}^{AB}$, $\hat{y}^{AB}$, $\hat{z}^{AB}$) defined by

$$\begin{aligned} \hat{x}^{AB} &= \alpha_x{}^A\, \hat{x}^A + \beta_x{}^A\, \hat{y}^A + \gamma_x{}^A\, \hat{z}^A \\ &= \alpha_x{}^B\, \hat{x}^B + \beta_x{}^B\, \hat{y}^B + \gamma_x{}^B\, \hat{z}^B \end{aligned} \tag{3}$$

and analogous expressions for $\hat{y}^{AB}$ and $\hat{z}^{AB}$.

X, Y and Z are the monomer fine-structure tensor eigenvalues, which are related to the fine-structure constants in the well known way ($D = -\frac{3}{2}Z$, $E = \frac{1}{2}[X - Y]$).

Thus, in principal it is possible to determine the dimer geometry from the measured values of both the fine-structure constants or the triplet sublevel depopulation rates, provided the model on which this estimate was based (strong coupling and incoherent triplet exchange) is correct. For the numerical determination of the direction cosines their orthonormality relations should be remembered.

To illustrate the procedure to determine these angles the results on bacteriochlorophyll [2–8] shall be briefly discussed in the following. Based on both optical spectroscopy [9, 10] and on magnetic resonance [13, 24] it has been argued that the reaction center in photosynthetic bacteria contains a pair of strongly interacting bacteriochlorophylls. Based on the monomer data, for instance of bacteriochlorophyll in THF ($D = (0.0238 \pm 0.0005)$ cm^{-1}, $|E| = (0.0069 \pm 0.0003)$ cm^{-1}, $k_x = (2287 \pm 280)$ s^{-1}, $k_y = (3321 \pm 572)$, $k_z = (661 \pm 74)$ s^{-1} [7]) one can attempt to explain the corresponding data in photosynthetic bacteria using the dimer concept outlined above. The fine-structure constants obtained by various authors on different systems in vivo differ only slightly centering around $D^* = (0.0188 \pm 0.0003)$ cm^{-1} and $|E^*| = (0.0032 \pm 0.0002)$ cm^{-1}. However, the decay rate constants reported by different authors [5, 7] on the same system are significantly different. We have arbitrarily choosen to use numbers given in Ref. [7] for the tentative determination of the configuration of the hypothetical dimer ($k_x{}^* = (2675 \pm 300)$ s^{-1}, $k_y{}^* = (3033 \pm 200)$ s^{-1} and $k_z{}^* = (1600 \pm 180)$ s^{-1}).

In order to get an optimum agreement between these experimental data and the numbers calculated for special dimer geometries we have varied the Euler angles ϑ, φ and ψ which transform the A-molecule principal axes into those of the B-molecule [25] in steps of 1°. It turns out that an optimum fitting of D^* and E^* can be obtained by choosing $\vartheta = (47 \pm 1)°$. It is not possible to determine φ and ψ unambiguously, if only the fine-structure constants are consulted. However by comparing the experimentally determined rate constants to those calculated using Eqs. (2) it is possible to estimate these angles as $\varphi = (10 \pm 10)°$ and $\psi = (65 \pm 10)°$ or vice versa. This arrangement yields $k_x{}^{AB} = (2250 \pm 350)$ s^{-1}, $k_y{}^{AB} = (2600 \pm 400)$ s^{-1} and $k_z{}^{AB} = (1500 \pm 200)$ cm^{-1} in good agreement with the quoted numbers for $k_x{}^*$, $k_y{}^*$ and $k_z{}^*$.

The angles given are in good agreement with the results of Clarke and coworkers [6, 7]. Contrary to our approach these authors used a coherent-exchange model which we are reluctant to apply because of the difficulties mentioned above. Thus it does not seem possible to decide whether the triplet exchange is coherent or incoherent by studying the dimer fine-structure and rate constants. However a fair estimate of the dimer geometry seems to be possible.

3. Determination of the Structure of the Proposed Dimers B4 and B5

Apart from the ESR lines discussed in Sect. 2 (B1, B2 and B3) in MTHF-samples, which are doped with chl b at very high concentrations, two additional systems B4 and B5 are observed. Their most striking feature is that the fine-structure constants D are considerably smaller than those of B1, B2 and B3 (between 10 and 20%). An obvious interpretation of this observation is to assign these lines to dimers of either of the three systems B1, B2 or B3. In order to test this tentative assignment we have calculated the possible dimer fine-structure constants for all possible combinations and geometrical arrangements using the procedure described in the previous section. It turns out that the only consistent assignment is that of B4 as a dimer of B3 and that of B5 as dimer of B2. The angles between the z-axes of the two constituents A and B of the dimer are 41° for B4 and 47° for B5.

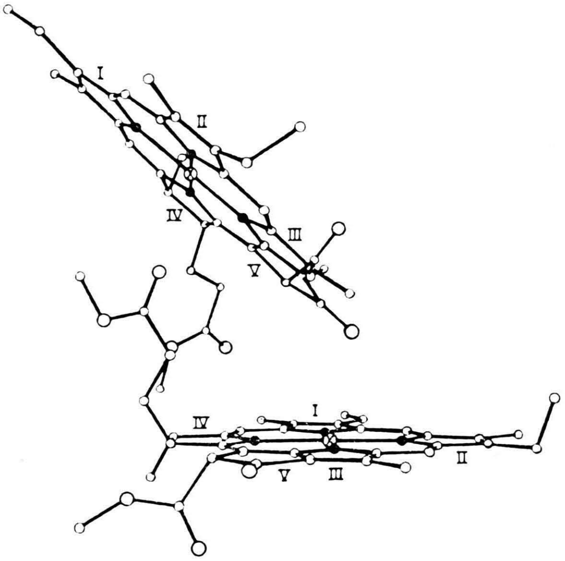

Fig. 1. Proposed structure of the chlorophyll dimer as obtained by Houssier and Sauer [10] based on investigations of circular dichroism and magnetic circular dichroism. Their model agrees well with the results obtained in this work.

These results are in excellent agreement with the results of Houssier and Sauer [9, 10]. Based on the comparison of the circular dichroism and the magnetic circular dichroism of several chlorophyll and protochlorophyll pigments they conclude that chl a and chl b as well as bacteriochlorophyll form dimers of the configuration indicated in Figure 1. The interplanar angle quoted by these authors is 45°.

Unfortunately it has not been possible to scrutinize this configuration using the kinetic constants. Since in high-concentration chl b samples in MTHF the observed ESR spectra are a superposition of 5 different spectra, we could not determine the individual rate constants for B4 and B5.

Investigations of the optical properties of the samples studied in this work (absorption, fluorescence and phosphorescence) [23] yield some additional support for the assignment presented in this work. These results will be published in a separate paper.

Acknowledgements

The authors are grateful to F. Drissler and Dr. H. Sixl for many valuable suggestions and discussions. Thanks are due to Dr. L. Schwan for help in writing the computer programs.

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