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The Dynamical Transition in Proteins of Bacterial Photosynthetic Reaction Centers Observed by Echo-Detected EPR of Specific Spin Labels

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Abstract. An echo-detected electron paramagnetic resonance (ED EPR) approach was used to study molecular dynamics in photosynthetic reaction centers (RCs) from *Rhodobacter sphaeroides* R26, employing the specific methanethiosulfonate spin label and 3-maliemido proxyl. ED EPR has recently been shown to be sensitive to so-called dynamical transition in disordered media, which is characterized by the transition from a harmonic-like librational motion of a molecule to an anharmonic one or to a stochastic wobbling motion. ED EPR line shapes studied over a wide temperature range reveal a sharp transition occurring above 180 K. The possible relation of the found transition to the temperature dependence of electron transfer reactions in RC is discussed.

1 Introduction

A sharp increase of the mean-squared dynamical displacement of atoms was detected in proteins between 200 and 230 K by neutron scattering [1–3] and Mössbauer absorption [4, 5]. It was also found for other biological systems, such as tRNA [6]. This increase is attributed to the change of the dynamical behavior of proteins from harmonic-like oscillations to anharmonic ones and is called a dynamical transition. It was demonstrated that this transition correlates with the onset of the measurable biochemical activities in biomolecules [7–9].

Several studies of the electron transfer in bacterial photosynthetic reaction centers (RCs) seem to indicate sudden changes of the reaction rates around 200 K [10–14]. One may suggest that this behavior may be related with the dynamical transition of the RC protein. However, such dynamical transition has not yet been

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explored for photosynthetic RC. In ref. 15 some indications were found that in the bacterial RC, glass transition occurs near 200 K. This conclusion was made on the basis of a study of the distances in the transient $P^+Q_A^-$ radical pair, employing out-of-phase electron spin echo spectroscopy.

Recently it has been demonstrated [16] that dynamical transition in glasses may be observed employing echo-detected electron paramagnetic resonance (ED EPR) of spin labels. In this experiment, a two-pulse echo sequence is applied. ED EPR line shapes are measured by scanning the magnetic field while keeping the time separation τ between two pulses fixed. Performing this experiment at different τ values provides a two-dimensional data set. The ED EPR line shape is nearly temperature- and τ-independent below the dynamical-transition temperature, while increasing the temperature results in a pronounced temperature and τ dependence of the ED EPR line shape, which marks the onset of anisotropic transverse spin relaxation. There exists strong evidence that this anisotropic relaxation is induced by fast low-amplitude orientational motion of the spin label. Comparison with the temperature dependence of neutron scattering data and employing some simple quantitative estimations provides the evidence that this onset may be explained by a transition from the harmonic librational motion to the anharmonic one or to fast stochastic wobbling motion [16] (it was impossible to make a choice between the last two options).

Previously, ED EPR of spin labels in biological objects was applied to study intracellular glass formation in living biological tissues (seed and pollen) [17] and to study glassy states in model phospholipid membranes [18]. However, the relation of the temperature and/or τ dependence of ED EPR line shape to the dynamical transition was not discussed in those works. (It is interesting to note that ED EPR data for phospholipid membranes [18] indicate a sharp increase near 200 K of the anisotropic relaxation rate.)

In the present work, we applied ED EPR to study the dynamical transition in site-specificly spin-labeled photosynthetic RC proteins. Two types of cysteine-specific spin labels were used: methanethiosulfonate spin label (MTSL) and 3-maliemido proxyl spin label. Previously, local molecular dynamics of spin-labeled RCs were studied using continuous-wave (CW) EPR at two microwave bands (X and D) [19].

2 Experimental

RCs of purple photosynthetic bacteria *Rhodobacter sphaeroides* R26 were isolated as described by Feher et al. [20]. RCs were solubilized in a buffer consisting of 10 mM Tris-HCl (pH 8.0), 1 mM EDTA and 0.025% lauryl- dimethylamine N-oxide. The spin label 1-oxyl-2,2,5,5-tetramethyl-Δ-pyrroline-3-methyl methanethiosulfonate was purchased from Toronto Research Chemical Inc. (Canada). The spin label 3-maliemido proxyl was obtained from Aldrich Chemical Co. The labeling of the RCs protein is described in detail in ref. 21. A typical EPR sample contained 66% (v/v) of glycerol to provide a transparent glass when freezing. Final concentration of spin labeled protein was about 70 μM. The ratio of bound spin label to RC was determined by CW EPR to be 0.9 ± 0.2 (for details see ref. 21). As was shown [21] the spin label is mainly attached to cysteine residue at position 156 on the H subunit of RC protein.

Electron spin echo measurements were carried out on an Elexsys E-680X/E-580E FT EPR spectrometer equipped with a dielectric cavity (Bruker ER 4118 X-MD-5) inside an Oxford Instruments CF 935 liquid-helium flow cryostat. Dead time caused by resonator ringing was about 100 ns. Durations of microwave pulses in the 90°-τ-180° pulse sequence were 12 and 24 ns, respectively. The repetition frequency was varied between 10 and 100 Hz depending on the temperature of the measurements, to provide full restoration of the magnetization between subsequent series of the pulses.

3 Results and Discussion

For both spin labels and at the whole temperature interval studied (60–220 K) CW EPR line shapes were found to be similar to those for solids. Molecular motion of spin-labeled RC protein results in a decrease of the total hyperfine splitting between the two outer components of the CW EPR spectra, because of motional narrowing. Figure 1 presents temperature dependence of the measured splitting.

Within the framework of the model of fast restricted angular oscillations (librations), the CW EPR line shape is determined by the same equations as a solid-like EPR spectrum, with the principal hyperfine interaction and *g*-factor values replaced by the motion-averaged counterparts [22]. The splitting between the two outer peaks in Fig. 1 corresponds with good accuracy to the $2\langle A_{ZZ} \rangle$ value (A_{ZZ} is the principal value of the hyperfine interaction tensor, the angular brackets mean the motional averaging, the molecular Z axis is perpendicular to the >NO plane). We consider a simple model of uniaxial librations in the >NO plane and denote α the angle of deviation from the equilibrium. Let us assume that the potential well is symmetric (the unharmonicity is small), so that $\langle \alpha \rangle = 0$. Then the motion-averaged A_{ZZ} value will be $\langle A_{ZZ} \rangle = A_{ZZ}^0 - (A_{ZZ}^0 - A_{\perp}^0)\langle \sin^2 \alpha \rangle$, where the superscript denotes data for a motionless molecule [22]. For a small amplitude of motion one may replace $\langle \sin^2 \alpha \rangle$ by $\langle \alpha^2 \rangle$. One can see in Fig. 1 a sudden drop of $\langle A_{ZZ} \rangle$ between 180 and 200 K, which may be explained within the framework of this model by increasing the motional amplitude α.

The validity of the librational model could be proved in ED EPR line shape studies. ED EPR spectra taken for MTSL at different temperatures are shown in Fig. 2. To exclude all isotropic relaxation processes that are expected to be field-independent, ED EPR spectra were normalized to the same value at the field position corresponding to the maximal amplitude. Since the ED EPR signal at different field positions is related to different nitroxide orientations relative to the external magnetic field, after such normalization the line shape is solely determined by the anisotropy of relaxation. One can see slight changes of spectral

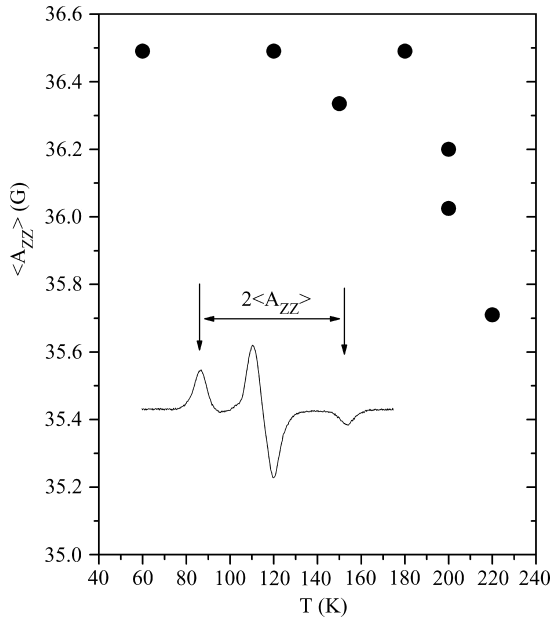

Fig. 1. Temperature dependence of the outer spectral splitting in CW EPR spectra measured for MTSL as shown in the inset.

shape between 100 and 160 K and pronounced changes of the ED EPR line shape in the temperature interval between 180 and 220 K.

The ED EPR line shape dependence on τ is presented in Fig. 3 for different temperatures. Below 190 K the line shape does not depend on τ, while above this temperature it does.

Some slight temperature dependence of the line shape seen between 100 and 160 K (Figs. 2 and 3) is probably related to the motion of methyl groups, which is known to manifest itself in electron spin echo of nitroxides at this temperature interval [23]. However, this motion must not influence the anisotropic relaxation, because of the absence of τ dependence at these temperatures (see Fig. 3). Therefore, motion of methyl groups probably results in partial averaging of the unresolved hyperfine interaction with methyl protons, which in turn influences the total line shape.

The changes of the line shape visible in Fig. 2 above 180 K and in Fig. 3 above 190 K are typical for low-amplitude librations [16, 18, 22, 24, 25]. The observed anisotropic relaxation is remarkably slower at the field positions corresponding to the canonical orientations of nitroxide (the outer spectral edges correspond to the orientation of spin label at which the molecular axis Z is parallel to the direction of the external magnetic field). This may be readily explained for the molecule performing a restricted orientational motion with a small amplitude. In that case, the Larmor frequency fluctuations are larger for the intermediate positions in the spectrum and smaller for positions corresponding to the canonical orientations [24, 25].

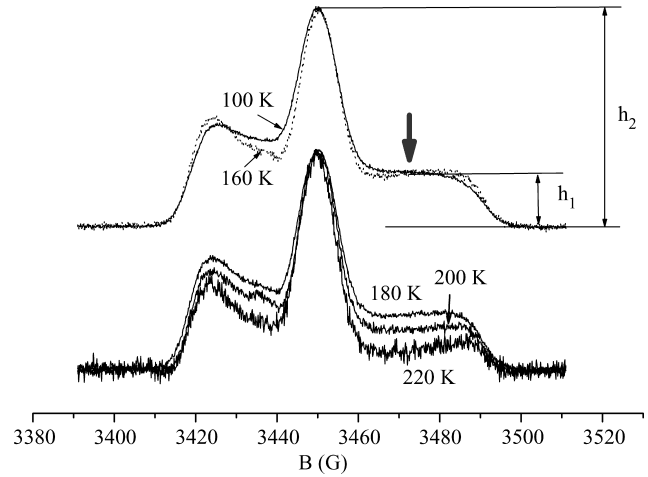

Fig. 2. ED EPR spectra of MTSL bound to RC protein taken at different temperatures. Spectra are normalized to the maximum amplitude. The bold arrow shows the field position at which the relative intensities (shown in Fig. 4) were measured.

Figure 4 presents the dependence of relative intensity taken at the spectral position indicated in Fig. 1, for two time intervals τ, 128 and 316 ns. For both

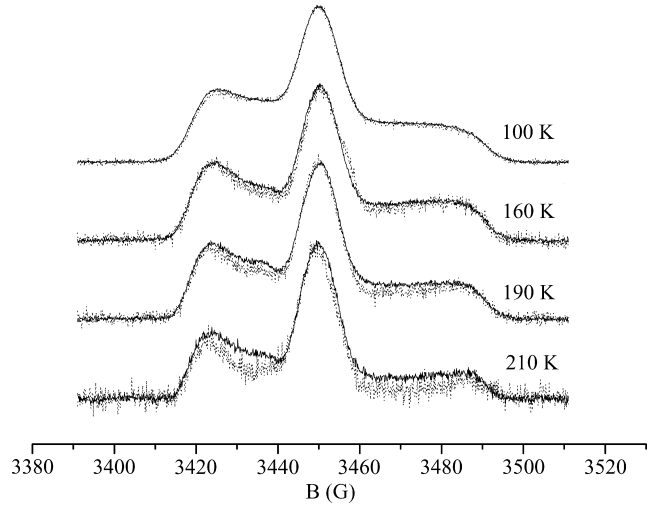

Fig. 3. ED EPR spectra for bound MTSL taken at different temperatures and for two time separations τ, 128 ns (solid line), and 316 ns (dotted line). Spectra are normalized to the maximum amplitude.

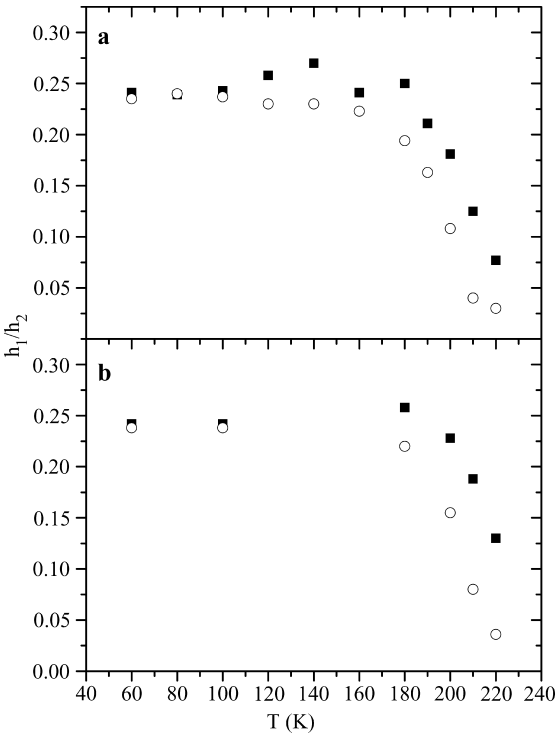

Fig. 4. Relative intensities in the spectra taken at the field position indicated by arrow in Fig. 2, as a function of temperature. **a** MTSL, **b** 3-maliemido proxyl. Closed squares, $\tau = 128$ ns, open circles, $\tau = 316$ ns.

spin labels one can see the slight increase of this intensity between 100 and 160 K and the sharp decrease above 180 K. The possible reason for the slight increase between 100 and 160 K was discussed above. The drop in the relative intensity above 180 K evidences the onset of anharmonic motion (or stochastic motion) [16]. For the greater τ value the effect is more pronounced, but the accuracy of measurement here is worse (see Fig. 3). The onset occurs for both labels at approximately the same temperature.

Note that the study of CW EPR line shapes performed in ref. 19 indicated the presence of motions of spin labels well below 180 K. In our opinion, this may be readily explained by harmonic librational motion at low temperatures, which is known to influence CW EPR line shape [16].

4 Conclusions

In line with the interpretation given in ref. 16, the onset of the temperature dependence of the ED EPR line shape is related to the dynamical transition from the harmonic librational motion of molecules to the anharmonic one or to a stochastic wobbling motion. From the results of the present work we may therefore conclude that the dynamical transition known near 200 K for proteins and other biomolecules [1–6] and also for model phospholipid membranes [18] occurs in photosynthetic RC protein as well and near the same temperature (180–200 K). This effect may be compared with the electron transfer reaction peculiarities which are known to occur near 200 K [10–14]. The electron transfer between Q_A (the primary acceptor quinone) and Q_B (the secondary acceptor quinone) is blocked at temperatures below 200 K [10]. The $P^+Q_A^-$ back reaction rate in aqueous solution was about 100 ms at 300 K and decreased to about 20 ms at 200 K [11]. Simulations of the temperature dependence of the $P^+Q_A^-$ back reaction by a model in which the back reaction is coupled to vibrational modes in the protein [12] showed that above 200 K the data could not be simulated with the model (see also ref. 13). The $P^+Q_A^-$ lifetime in a set of mutants with different P/P^+ redox potentials was found to suddenly change around 200 K [14].

One may suppose that these features of the temperature dependence of electron transfer reactions are related with the dynamical transition in the RC proteins. The detailed mechanism of this relation could be the subject of future investigations.

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