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Conformation transition in the protein of a photosynthetic reaction center observed at the nanometer range of distances at cryogenic temperatures

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Abstract

Electron spin echo (ESE) spectroscopy is applied to study magnetic dipolar interaction between electron spins in the transient $P^+Q_A^-$ radical pairs (P is the primary donor, a bacteriochlorophyll dimer, and Q_A is the primary quinone acceptor) and the $^3PQ_A^-$ triplet-radical pairs in bacterial photosynthetic reaction centers of *Rhodobacter sphaeroides* R26. Distance separation in both pairs is about 29 Å. A well-resolved reversible conformational transition of the reaction center protein holding the P and Q_A cofactors was observed between 13 and 20 K. This transition results in a narrowing of the distribution of protein conformations with decreasing temperature, with the width of distance distribution between P and Q_A dropping from ca. 4 Å at 20 K to ca. 1 Å at 13 K. This transition implies the existence of low barriers in the protein energy landscape and the presence of cooperatively rearranging domains of the size of several nm both in the protein and in the surrounding glassy environment.

1. Introduction

Protein dynamics at low temperatures nowadays is intensively studied using different experimental approaches, such as spectral hole burning and photon echo techniques [1–4], infrared vibrational echo technique [5,6], single molecule spectroscopy [7], Mössbauer spectroscopy [8] and many others. There are a lot of indications that protein conformational motions persist even at cryogenic temperatures. This motion is determined by the so-called energy landscape, the complex hierarchy of energy levels associated with the manifold of structures produced by the set of amino acids which form the protein [9]. These data have been obtained for sites containing co-factors, such as the heme group in heme protein, Zn-protoporphyrin in myoglobin, etc. The dynamics probed by these approaches reflect local motion, at the atomic scale, although it may be related with global conformational motion [6]. In this work we present results of an electron spin echo (ESE)

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spectroscopic study of radical pairs. The method used in this study [10] is capable to directly observe structural changes that occur on the nanometer scale.

In our study we use bacterial photosynthetic reaction centers (RCs). They are integral membrane proteins whose structure was solved to the atomic level by X-ray diffraction. Absorption of light by isolated RCs results in the formation of a transient radical pair $P^+Q_A^-$, where P denotes the primary electron donor, a bacteriochlorophyll dimer, and Q_A is the primary electron acceptor quinone (for review see, e.g. [11]). These cofactors are held in a well-defined relative position by the protein. The distance between P and Q_A is about 29 Å [11]. At low temperature the radical pair recombines in about 30 ms and the sample is restored to its initial condition. With Q_A chemically pre-reduced to the Q_A^- state, the transient $^3PQ_A^-$ pair is formed under light excitation, where ^3P is the triplet state of the primary donor. After decay of ^3P to the ground state the sample is again restored to its initial condition. In both cases, the populations of spin states of the pairs are not in thermal equilibrium but are spin-polarized.

Using ESE spectroscopy, distances can be obtained with an accuracy better than 0.4 Å [12–14]. For the $P^+Q_A^-$ radical pair, a pulse sequence consisting of two microwave pulses is employed, pulse1–τ–pulse2–τ–echo, forming a primary out-of-phase ESE at the time moment 2τ [10,12,13]. For the $^3PQ_A^-$ triplet-radical pair, a three-pulse sequence is employed, pulse1–τ–pulse2–T–pulse3–τ–echo, forming a stimulated out-of-phase ESE [15]. The pulse sequences are applied with a short delay after the light flash. As τ is varied, the echo intensity is strongly modulated (ESE envelope modulation or ESEEM). Sine Fourier transformation of the ESEEM provides a so-called Pake resonance pattern which is determined by the magnetic dipole–dipole interaction between the two spins of the pair,

$$d = -\frac{1}{2\pi}\frac{3}{2}\frac{\gamma^2\hbar}{r^3}(3\cos^2\theta - 1),$$

where γ is the gyromagnetic ratio, r is the distance between two the spins of the pair, θ is the angle between the vector connecting the two partners of the pair and the magnetic field of spectrometer. For a distance of around 30 Å, d is about 3 MHz (for $\theta = \pi/2$). This is higher than the typical transverse spin relaxation rate in organic solids, which damps the ESEEM pattern, and therefore, distance r can be determined in these experiments.

The two characteristic frequencies of the Pake resonance pattern, $f_\perp$ and $f_\parallel$, correspond to the singularity at the perpendicular orientation ($\theta = \pi/2$) and to the edge of the frequency spectra attained at the parallel orientation ($\theta = 0$), respectively:

$$\begin{aligned} f_\perp &= \pm\frac{1}{2\pi}\frac{\gamma^2\hbar}{r^3} \\ f_\parallel &= \mp\frac{1}{\pi}\frac{\gamma^2\hbar}{r^3} \end{aligned} \tag{1}$$

The plus and minus correspond to two sets of these frequencies. In (1) spin exchange interaction between two electrons is neglected, as it is known to be negligibly small for the $P^+Q_A^-$ pair [12].

In the present work we investigate the temperature dependence of the Pake resonance pattern for the RCs of *Rhodobacter* (*Rb.*) *sphaeroides* R26. Previous investigations performed on the $P^+Q_A^-$ pairs in a temperature range between 15 and 200 K have shown that structural rearrangements take place near and below 80 K [12]. Below this temperature the protein exists in a set of conformations. Lowering the temperature results in a broader distribution of distances between P and Q_A, approaching ~4 Å at 15 K. Above 80 K the Pake lineshape becomes much narrower. However, it was not possible to decide whether this narrowing is induced by a narrowing of the static conformational distribution or whether it is a spectroscopic narrowing of the Pake lineshape due to fast exchange between conformations. Also, a controversial point was the possible temperature dependence of the transverse relaxation time T_2, which would influence the interpretation.

In this paper we report another transition occurring around 15 K that results in narrowing of the Pake lineshape as temperature decreases. As lowering the temperature slows down any motion, a definite conclusion may be that this transition results in a more ordered state at low temperatures. By employing the triplet-radical pairs $^3PQ_A^-$,

the problem of T_2 is also lifted since the ESEEM pattern is obtained from the Q_A^- radical with known T_2.

2. Experimental

Reaction center isolation from *Rb. sphaeroides* R26 and sample preparation was done as in [15]. The paramagnetic Fe^{2+} ion embedded in the membrane in the vicinity of the cofactors was replaced by diamagnetic Zn^{2+}, to avoid fast relaxation induced by the iron ion. Samples contained 66% (v/v) glycerol, to provide a transparent glass when freezing the sample. Experiments 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. As a light source for sample irradiation inside the ESE cavity a Continuum Surelite I laser was used. The excitation wavelength was 532 nm. The repetition rate of the laser flashes of ca. 4 ns duration was 10 Hz, or, optionally, 3 Hz. Microwave pulses were delayed after the laser flash by 1 μs. To obtain the $^3PQ_A^-$ triplet-radical pair, the sample was used with chemically reduced Q_A. The phase of the microwave pulses was adjusted employing the ESE signal of Q_A^- in the dark.

3. Results

Fig. 1a shows time-domain traces of the out-of-phase ESEEM for the $P^+Q_A^-$ radical pair (two-pulse primary echo), and Fig. 1c shows those for the $^3PQ_A^-$ triplet-radical pair (pre-reduced sample, three-pulse stimulated echo). The out-of-phase ESEEM is induced by magnetic dipole–dipole interaction between the two spins in the pair. Fig. 1b shows the in-phase ESEEM for the Q_A^- stable radical (no light irradiation is applied). As this radical is in a thermal equilibrium, only electron–nuclear interactions contribute to ESEEM [16]. (This kind of ESEEM is suppressed in spin-polarized radical pairs [17].)

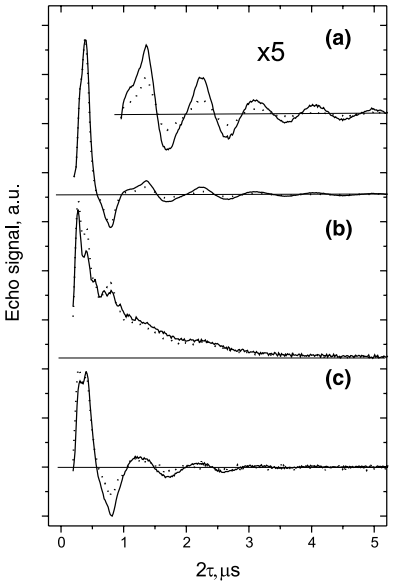

Fig. 1. ESEEM obtained at 13 K (solid line) and at 20 K (dotted line). (a) $P^+Q_A^-$ transient radical pair appearing after laser flash, two-pulse ESE, signal is out-of-phase; (b) Q_A^- stable radical, three-pulse stimulated ESE, signal is in-phase (no light illumination is applied); (c) $^3PQ_A^-$ transient triplet-radical pair appearing after laser flash, three-pulse stimulated ESE, out-of-phase signal. In the three-pulse experiments delay τ between first and second pulses is scanned while the delay T between second and third pulses is fixed to 10 μs. Traces are shifted arbitrarily along the vertical axis, the zero horizontal line is given for all cases.

In Fig. 1 results are given for two temperatures, 13 and 20 K. One can see that out-of-phase ESEEM is noticeably temperature-dependent. At 13 K oscillations last for much longer time than those at 20 K, which is clearly seen in the insert of Fig. 1a. This difference was well reproduced for different samples and for many freezing-down–warming-up cycles.

No noticeable temperature dependence was found between 4.2 and 13 K (data not given). Above 20 K, the data obtained depended on temperature and reproduced those published for this system previously [12].

The ESEEM of the $P^+Q_A^-$ radical pair at low temperatures was found to depend slightly on the repetition rate of the laser flashes. This may be related to populating the spin sublevels of the radical pair by microwave pulses so that at low

temperatures and high repetition rate the pair does not recombine before the next pulse sequence is applied [18]. Data in Fig. 1a are given for the repetition rate of 3 Hz (the observed difference between two temperatures was found larger than that for 10 Hz, so the decreasing repetition rate increases the effect). Such mechanism is not operative for the $^3PQ_A^-$ triplet-radical pair and for this pair we indeed did not observe any ESEEM dependence on the repetition rate.

Fig. 2 shows the sine Fourier transform of the out-of-phase ESEEM for the $^3PQ_A^-$ triplet-radical pair (data in Fig. 1c). Prior Fourier transformation, in the dead time the data were restored using a parabolic interpolation (at $t = 0$ the signal intensity is known to be zero, see [12] for details), and zero-filled. One can see that at 13 K the lineshape is noticeably sharper than that at 20 K. For the $P^+Q_A^-$ radical pair Fourier transform of ESEEM (data in Fig. 1a) gives an analogous result.

The observed temperature dependence may be thought to be due to a stepwise change of the transverse relaxation time, T_2, between 13 and 20 K.

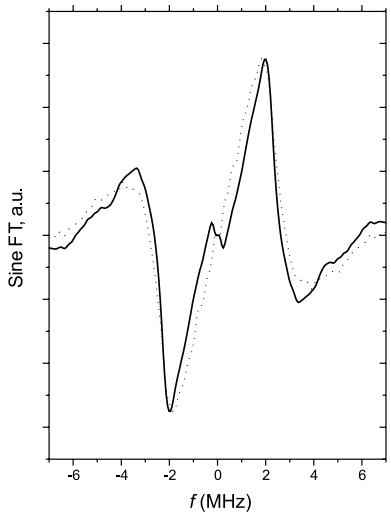

Fig. 2. Fourier transform of ESEEMs given in Fig. 1c for 13 K (solid line) and for 20 K (dotted line). The result is a Pake resonance pattern, which has an antisymmetric lineshape due to spin polarization. The singularities correspond to the perpendicular orientation of the pair, the edges correspond to the parallel orientation. The feature near zero frequency (solid line) is an artifact.

Indeed, if T_2 at 20 K is shorter than at 13 K, the oscillation pattern at 20 K will be damped resulting in a broadening of the Pake lineshape. However, we did not observe such change of T_2 for the in-phase ESEEM of Q_A^- radical in the dark – see Fig. 1b (and also see below). As for the $^3PQ_A^-$ triplet-radical pair the same radical is under study (^3P has a very broad resonance line and therefore is almost unaffected by microwave pulses [15]), one may expect that T_2 does not change for this pair as well. (Magnetic dipole–dipole interaction between two spins in the pair hardly may influence T_2.) Moreover, we did not observe a noticeable temperature dependence of the in-phase ESEEM for the Q_A^- radical in the entire temperature range studied, from 4.2 to 60 K (data not given).

In addition we have studied the out-of-phase ESEEM at temperatures between 13 and 20 K, in order to determine precisely the transition temperature. However, we observed only continuous changes of the ESEEM pattern (data not given). Thus, it appears that the transition is not sharp but extends over that temperature range.

4. Discussion

In our opinion, the only way to explain the observed temperature dependence of ESEEM is to assume a narrowing of the distribution of conformations below 13 K. This may take place if barriers in the energy landscape allow these conformational transitions and if there is a freedom for the protein, that is embedded in a rigid glycerol glass, to rearrange itself at the nanometer range of distances.

Low-height energy barriers are known for proteins from other studies. E.g., a photon echo experiment on myoglobin [2] has revealed that the energy landscape contains barriers of 0.2, 0.4, and 0.6 kJ/mol (24, 48, and 72 K in the temperature units). These barriers may allow conformational transitions for proteins at cryogenic temperatures. From the other hand, a lot of indications have appeared recently in literature that glasses possess a freedom for cooperative rearrangement at the nanometer scale of distances [19,20] (a so-called dynamical heterogeneity).

Fig. 3 shows simulations of the Pake spectra. $P^+Q_A^-$ and $^3PQ_A^-$ pairs are spectroscopically weakly coupled, in the sense that $2\pi d \ll \Delta\omega$, where $\Delta\omega$ is the difference of the Larmor frequencies of the two spin partners in the pair [21]. For this case, a simple convolution of the pure Pake lineshape with the Lorentzian lineshape determined by transverse relaxation may be employed [14,21],

$$P(f) \propto \int \sin\theta \, \mathrm{d}\theta \frac{1}{\pi} \times \frac{T_2}{1 + T_2^2(2\pi f - (\gamma^2\hbar/r^3)(3\cos^2\theta - 1))^2} \tag{2}$$

which must be antisymmetrized, $P_0(f) = P(f) - P(-f)$, for a spin-polarized pair. Further, we assume that distances are distributed by some function, $n(r)$. We optionally employed Gaussian or restricted Lorentzian distributions. The latter,

$$n(r) \propto \left(1 + \frac{(r - r_0)^2}{\delta^2}\right)^{-1},$$

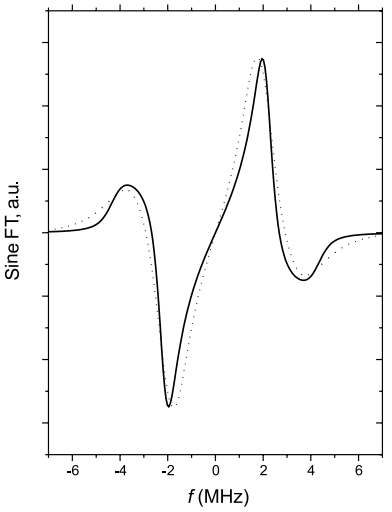

Fig. 3. Computer simulation of the Pake spectrum, using convolution with a Lorentzian lineshape (Eq. (2)). Distances in the pair are distributed by a restricted Lorentzian function (see text). Parameters of calculation: $T_2 = 1$ μs and $\delta = 0.5$ Å (solid line), $T_2 = 0.9$ μs and $\delta = 2$ Å (dotted line), $r_0 = 28.9$ Å for both cases.

with $|r - r_0| < 2\delta$, turned out to fit the experimental lineshape better. Eq. (2) was averaged over this distribution. Values of $T_2 \cong 1$ μs for 13 K and $T_2 \cong 0.9$ μs for 20 K were obtained by exponential fitting the data in Fig. 1b. The parameters of the distance distribution were obtained by fitting data in Fig. 2: $r_0 = 28.9$ Å for both cases, and $\delta = 0.5$ Å (solid line), $\delta = 2$ Å (dotted line). There results are shown in Fig. 3. One can see that the agreement between calculations in Fig. 3 and the experiment in Fig. 2 is rather good. Some details do not coincide probably because the true distribution differs from the one used in simulations.

Thus, our simulations seem to indicate that the distribution width 2δ drops from about 4 Å above 20 K to about 1 Å below 13 K. This drop certainly reflects a conformational transition occurring in this temperature range. Our results also show that the transition is well-reproduced and fully reversible.

At temperatures above 20 K the protein probably does not exist in one fixed conformation but a dynamical equilibrium between different conformations probably takes place. Therefore one may think that the protein behaves like a highly viscous liquid above 20 K. In particular, the temperature hysteresis found previously [12] may be expected for an extremely highly viscous liquid. Taking also into account the reversibility of the transition we may point out that it resembles a transition between a liquid (of very high viscosity) and a crystal.

5. Conclusions

Out-of-phase ESE spectroscopy of spin-correlated radical pairs in photosynthetic RCs provides a very sensitive tool to study conformational changes in the proteins of RCs. The unique feature of these studies is the possibility to detect changes at the nanometer range of distances (around 3 nm in this work), with an accuracy better than 0.1 nm.

In this work we found a reversible conformational transition of the protein holding the P and Q_A cofactors occurring between 13 and 20 K. Transition results in narrowing of the distribution

of protein conformations with decreasing temperature, with the width of distance distribution between P and Q_A dropping from ca. 4 Å at 20 K to ca. 1 Å at 13 K.

The possibility of this low-temperature transition implies that the energy landscape of the protein contains very low barriers. As a result, the rearranging of a large protein molecule at cryogenic temperatures occurs, to attain a more stable thermodynamic state. Also, cooperatively rearranging domains of the size of several nm must exist in the surrounding glassy environment, to facilitate this rearrangement.

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