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TEMPERATURE DEPENDENCE OF THE CHEMICALLY INDUCED DYNAMIC ELECTRON POLARIZATION IN GREEN PLANTS AND ALGAE

The temperature dependence of the chemically induced dynamic electron polarization has been studied in the chloroplasts of Anacystis nidulans and Scenedesmus obliquus. The spectrum of photosystem I observed is different from that of $P700^{+}$ with respect to shape and the g value, and points to the presence of a non-chlorophyll organic radical in the reaction centre of photosystem I.

Research on photosynthetic organisms has long provided a meeting ground for scientists from a variety of disciplines. The photosynthetic systems has been able to challenge and interest solid-state physicists, chemists and biologists by presenting them with a multitude of physical and biological processes ranging from exciton transfer in pigment array to the growth of forests. In this diverse field it is therefore not surprising that a remarkable range of physical techniques has been employed to elucidate various mechanistic aspects of photosynthetic energy storage.

In the past decade the application of magnetic resonance techniques has contributed much to our still imperfect understanding of these processes [4]. Its use in the study of photosynthesis is perhaps the most rewarding application of electron paramagnetic resonance (EPR) on material of biological relevance. Practically all aspects of this technique are represented in one or another form in the rapidly growing body of literature on this subject. Experiments exploiting the phenomenon of chemically in-

duced dynamic electron polarization (CIDEP) have been extraordinarily useful in photosynthetic research in the past few years [3, 4, 5].

Spin polarized, non-Boltzmann, or historically called CIDEP, EPR spectra result if a chemical reaction has a preference for one of the spin states of the products of the reaction. Two mechanisms for the generation of the nonequilibrium spin distribution have been identified in chemical systems, the radical pair and triplet mechanisms [1, 9]. A radical pair is simply two radicals whose electron spins are correlated with respect to each other; i.e., the relative orientation of the two electron magnetic moments is not random. This correlation can exist in a single molecule before radical formation or can be produced by spin-selective reactions between independently generated radicals. A single molecule with two unpaired electrons that interact strongly is usually called a triplet rather than a radical pair.

Photosynthesis begins when a photon is absorbed by a pigment molecule embedded in a biological membrane [2] (the chloroplast membrane of green plants or the cytoplasmic membrane of photosynthetic bacteria), promoting it from the ground state to an excited state. The energy is rapidly transferred to a chlorophyll (or bacteriochlorophyll in the case of photosynthetic bacteria) in a specialized chlorophyll-protein complex called the reaction center. In this excited state the pigment molecule is an extremely strong reductant, and an electron is lost to an acceptor molecule strategically placed nearby. The oxidized chlorophyll and reduced acceptor then rapidly react with secondary electron donors and acceptors to separate the charges and stabilize the system against recombination losses.

The first step in the spin polarization process is radical pair formation. In the case of photosynthetic systems, the radicals are oxidized and reduced species produced by the photochemical electron-transfer reaction. The two electron spins were highly correlated just before the reaction, and the chemical reaction preserves this correlation. Thus an excited singlet produces a singlet radical pair and an excited triplet produces a triplet radical pair. If the radicals are far enough apart, the individual spin vectors are free to precess about the field direction

at a frequency determined by the electron g factor and nuclear state of the radical. In the presence of a magnetic field and the absence of any exchange coupling between the two radicals, only the singlet (S) and middle triplet (T_o) levels are mixed. The spin Hamiltonian can be divided into two parts, one of which gives the frequency of $S \rightarrow T_o$ mixing [9].

$$\omega_{ab} = \hbar^{-1}\left[\frac{1}{2}\beta H_o(g_1 - g_2) + \frac{1}{2}\sum_n A_{1n}M_{1n} - \frac{1}{2}\sum_m A_{2m}M_{2m}\right] \quad (1)$$

In Eq. 1 ω_{ab} represents the difference in angular precession frequency of the two electrons, $\hbar$ is Planck's constant divided by 2π, β is the Bohr magneton, H_o is the applied magnetic field, g_1 and g_2 are the electronic g factors of the two radicals, and A_{1n} (or A_{2m}) are the isotropic hyperfine coupling constants of nucleus n (or m) on radical 1 (or 2) with magnetic quantum number M_{1n} (or M_{2m}). The simple $S \rightarrow T_o$ mixing process described above can account for some of the CIDEP observations in photosynthetic systems.

Doublet polarization requires both $S \rightarrow T_o$ mixing and an exchange interaction between the two radicals. The $S \rightarrow T_o$ mixing and exchange can either occur simultaneously on a single radical encounter or sequentially on two encounters. The doublet polarization (excess of upper spin state) of radical pair can generate in a single encounter [9]:

$$\rho = \frac{2\omega_{ab}J}{\omega_{ab}^2 + J^2}\sin^2(\omega t) \quad (2)$$

where $2J = E_s - E_t$ is the singlet-triplet splitting. In diffusing systems, due to the rapid Brownian motion of the molecules ($\approx 10^{-12}$s) this condition is not satisfied for a long enough time for significant polarization to develop in this manner. The fixed geometry of the photosynthetic reaction center effectively precludes free diffusion and multiple encounters of the radicals but is ideal for development of single-encounter polarization via single-triplet mixing and exchange interaction, if the radical separation is appropriate.

Materials and methods

Whole cells of the algae Anacystis nidulans and Scenedesmus obliquus were investigated, the cultures being maintained in our laboratory. Scenedesmus obliquus was cultivated in deuterated growing medium in a result 99% deuterated cells were used for experiments. Whole chloroplasts were prepared from market spinach by the procedure of W h a t l e y and A r n o n [11] and contein about 5 mg/ml of chlorophyll. Chloroplasts in a medium of 50% glycerol and 50 mM Tris at pH 8 were oriented in a 2.4 T magnetic field and stored in the frozen state at 77 K for subsequent EPR measurements. There were no exogeneous redox agents added to the samples. Flash photolysis measurements were carried out as described previously [5, 6].

Results

A single deuteron gives a triplet (instead of a doublet in proton case) hyperfine pattern. However, if all other factors are identical, the deuteron splitting is only one-seventh that of the proton. Because the EPR line widths are reduced in deuterated samples we have used deuterated algae in order to obtain better signal-to-noise ratios for kinetics taken without magnetic field modulation.

Examples of time-resolved EPR spectra for deuterated Scenedesmus obliquus in $0^{\circ}C$ are presented in Fig. 1. The shape of the field profils and g-factor region are identical to those obtained at liquid nitrogen temperature [7] and room temperature also [12] but the rate of the decay of transient signal is remarkably faster in higher temperatures.

The shape of the field profile at the beginning of the decay is also similar to that obtained by spin echo method [10]. In Fig. 1 a and b four stages of field profile in time span available for our direct detection system are shown. Changes of the shape of field profiles in time is an argument in favour of presence of more than one component with different rate of the decay. The last field profile (300 μs) is identical with steady

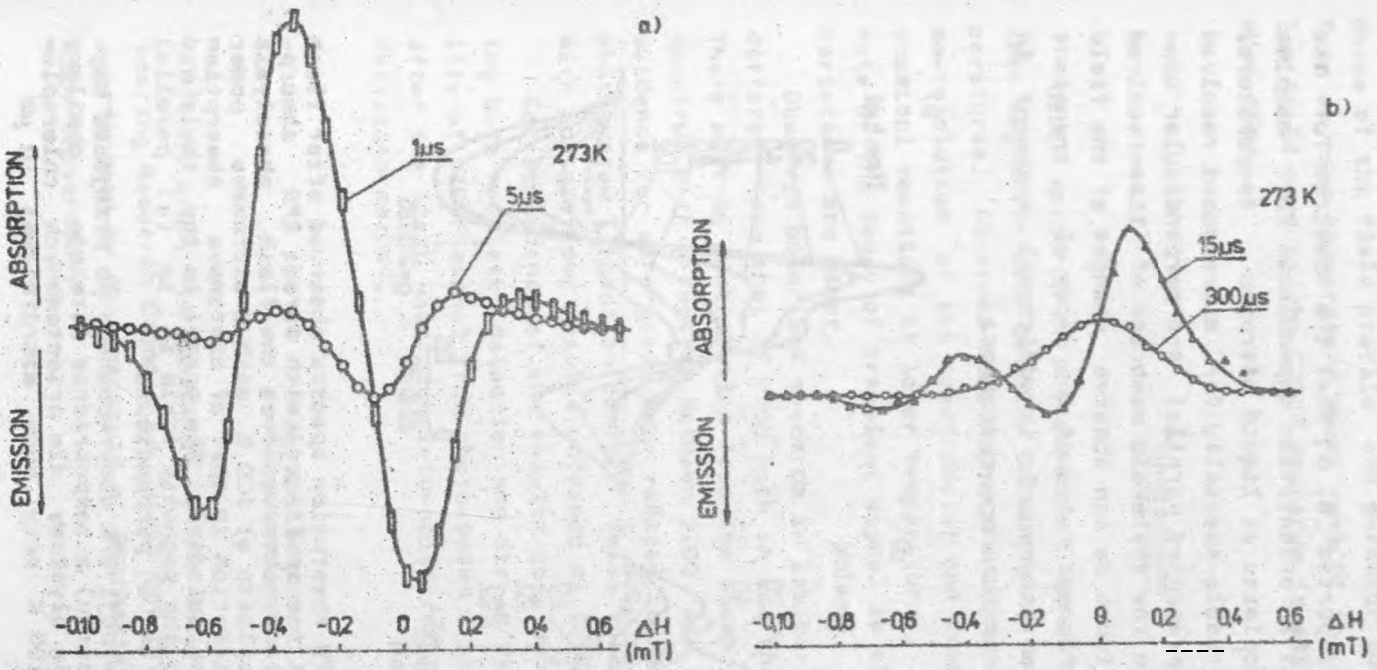

Fig. 1. Time-resolved EPR spectra observed from flash photolysis experiments with the direct absorption detection system for whole cells of the 99% deuterated algae Scenedesmus obliquus. The microwave power was 1 mW at 9.2 GHz, temperature 273 K. Field profiles in part (b) are 5x expanded in vertical direction in comparison to part (a)

Czas rozkładu widma EPR obserwowanego eksperymentu z fotolizą błyskową z bezpośrednim systemem detekcji absorpcji dla całkowitych komórek w 99% zdenaturowanych alg Scenedesmus obliquus. Moc mikrofalowa 1 mW przy 9,2 GHz, temperatura 273 K. Obszar profilu w części (b) rozszerzony 5x w kierunku pionowym w porównaniu do części (a)

state signal I in terms of the shape, g-factor value and half--width of the spectrum.

We have previously reported [6, 8] that the spectrum of the CIDEP transients exhibit an orientation dependence for samples of oriented whole chloroplasts at liquid nitrogen temperature. This anisotropy is remarkable specially for microsecond resolved spectra. In Fig. 2 are presented parallel and perpendicular components with respect to the thylakoid membrane of time-resolved EPR spectra. In this figure we can observe changes of the field profiles in time and different rates of the decay of transient signal for this two orientations. It is additional argument in favour of presence of more than one component.

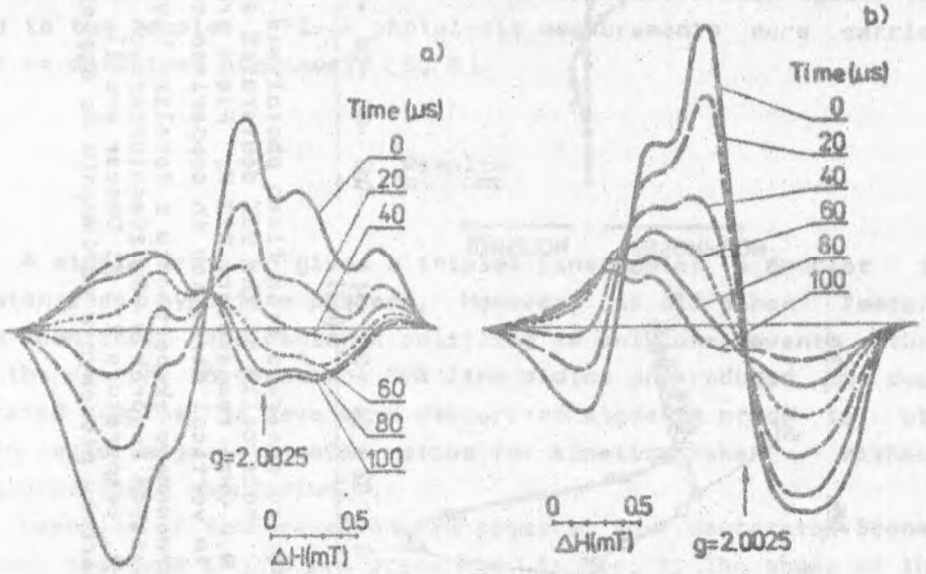

Fig. 2. Time-resolved EPR absorption spectra observed after flash excitation (time is written in figure) with direct EPR absorption detection of transients observed from the flash photolysis of oriented whole chloroplasts at 100 K with a microwave power of 10 μW. The upward direction is that of microwave absorption and downward is microwave emission. The normal to the thylakoid membrane with respect to the magnetic field is in (a) parallel and in (b) perpendicular

Czas rozkładu widma absorpcji EPR obserwowanego po przejściu błysku (czas wpisany na rysunku) z bezpośrednią detekcją chwilową absorpcji EPR z fotolizą błyskową dla orientowanych chloroplastów całkowitych w 100 K przy mocy mikrofalowej 10 μW

At higher temperatures rate of the decay is faster, but the shape of the field profile and g-factor span are the same [12]. Even at room temperature where rate of the decay is very fast, low field part of the CIDEP spectrum is stil present in microsecond time.

Conclusions

CIDEP spectrum of photosystem I appears generally within the same magnetic-field profile between room and liquid helium temperatures. Observed deviations are caused mainly by limited time-resolution of the spectrometer and frozen state of secondary chemical reactions at lower temperatures. In deuterated samples rate of the decay of transient signal is slower and temperature variations are minor.

Observed polarized spectrum in its early stage is completely different from that of $P700^+$ both in the shape and g-factor span. There must be contribution from the acceptor radical to this spectrum with g-factors between 2.00 and 2.01. Thus, there is evidence for an organic free radical (beside chlorophyll) in the photosystem I reaction center. These conclusions are consistent with our previous results obtained at low temperatures [6-8].

Correspondence of the results obtained in EPR experiments using both modulated detection and direct detection supports reality of CIDEP signal from photosystem I observed in shorter times after the flash when signal-to-noise ratio is better for direct detection method.

Acknowledgement

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REFERENCES

[1] Adrian F. J., Rev. Chem. Intermediates, 3, 3 (1979).
[2] Clayton R. K., Photosynthesis: Physical mechanisms and chemical patterns, Cambridge (1980).
[3] Dismukes C., McGuire A., Friesner R., Sauer K., Rev. Chem. Intermediates 3, 59 (1979).
[4] Hoff A. J., Physics Reports 54, 75 (1979).
[5] Manikowski H., McIntosh A. R., Bolton J. R., [in:] Photosynthetic solar energy conversion and storages, ed. J. Poskuta, Warsaw (in press).
[6] McIntosh A. R., Manikowski H., Bolton J. R., J. Phys. Chem. 83, 3309 (1979).
[7] McIntosh A. R., Manikowski H., Wong S. K., Taylor C. P. S., Bolton J. R., Biochem. Biophys. Res. Commun. 87, 605 (1979).
[8] McIntosh A. R., Manikowski H., Bolton J. R., [in:] Photosynthesis II. Electron transport and photophosphorylation, ed. G. Akoyunoglou Philadelphia (1981).
[9] Muus L. T., Atkins P. W., McLauchlan K. A., Pedersen J. B., [in:] Chemically induced magnetic polarization, eds. Reidel, Dordrecht(Holland), 1977.
[10] Thurnauer M. C., Bowman M. K., Norris J. R., FEBS Letters 100, 309 (1979).
[11] Whatley F. R., Arnon D. I., Methods Enzymol. 6, 308 (1963).
[12] Sent for publication.

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ZALEŻNOŚĆ TEMPERATUROWA DYNAMICZNEJ POLARYZACJI ELEKTRONOWEJ INDUKOWANEJ CHEMICZNIE U ROŚLIN WYŻSZYCH I GLONÓW

Badano zależność temperaturową dynamicznej polaryzacji elektronowej indukowanej chemicznie w chloroplastach Anacystis nidulans i Scenedesmus obliquus. Obserwowane widmo fotosystemu I jest różne od widma $P700^+$ co do kształtu i wartości g i wskazuje na obecność niechlorofilowego rodnika organicznego w centrum reakcyjnym fotosystemu I.