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Detection of deuterium nuclei in the immediate surroundings of P700 centers of plant photosynthesis by electron spin echo modulation

S.A. Dikanov, A.V. Astashkin, Yu.D. Tsvetkov, M.G. Goldfeld* and A.G. Chetverikov+

*Institute of Chemical Kinetics and Combustion, Novosibirsk 630090, *Institute of Chemical Physics, Moscow 117977* and +*Timiryazev Institute of Plant Physiology, Moscow 127276, USSR*

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A comparative analysis of the $P700^+$ electron spin echo modulation in preparations containing normal and heavy water has permitted the detection of the deuterium resulting from isotopic substitution in the immediate surroundings of the P700 centers. The contribution to the modulation is made by a single nucleus coupled with the unpaired electron with the anisotropic hyperfine interaction $T_\perp = 0.4$ MHz.

Photosynthesis; Photosystem I; Primary electron donor; Special pair; Electron spin echo; Hyperfine interaction

1. INTRODUCTION

The role of water in light-induced charge separation and recombination in photosynthesis reaction centers has been considered in at least three aspects. In highly hydrated systems (over 30% water by wt) water provides some conformational mobility of membrane proteins [1,2]; a smaller fraction of the water is likely to provide molecular relaxation in the immediate surrounding of the electron donor-acceptor pair, thus decreasing the energy of the excited complex and inhibiting recombination of the primary separated charges [3–6]. Finally, a few water molecules (1–2 per reaction center) are located within a special chlorophyll dimer in the reaction center [7,8]. Speculations on the water site in the chlorophyll dimeric center rest on theoretical considerations and on similarities between the P700 reaction center and hydrated chlorophyll aggregates in vitro. This very small fraction of the bound water in photosynthetic membranes has remained till now completely silent in spectroscopy of any kind. It is not known if these tightly and specifically bound water molecules could exchange their protons with bulk water outside the membrane.

Here, electron spin echo envelope modulation (ESEEM) spectroscopy [9,10] is employed for detecting deuterium nuclei in the immediate surroundings of the P700 centers after H_2O-D_2O exchange in freeze-dried preparations of leaf tissue of higher plants.

Correspondence address: S.A. Dikanov, Institute of Chemical Kinetics and Combustion, Novosibirsk 630090, USSR

2. MATERIALS AND METHODS

Freeze-dried preparations of leaf tissue of 2-week-old plants of wheat or horse-bean (*Vicia faba* L.), containing 12–15% H_2O or D_2O by wt, were obtained essentially as in [5,6]. To this end completely dehydrated preparations (water content $<2\%$) were incubated for a short time ($\sim$ 5 min) and then freeze-dried in the dark down to 12–15% hydration.

Chloroplasts were obtained from 2-week-old plants of *V. faba* as described in [11], in a medium containing 0.4 M sucrose, 0.01 M NaCl and 0.05 M

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Tris-HCl (pH 7.8). The preparation was frozen and stored in liquid nitrogen. Before the experiment the chloroplasts were thawed, centrifuged and twice resuspended in the same media prepared with H_2O or D_2O.

The P700 centers were oxidized under light while frozen in liquid nitrogen. The experiments have been performed on an X-band ESE spectrometer [12] utilising a two-pulse technique.

3. RESULTS AND DISCUSSION

Fig.1 represents the $P700^+$ primary ESE signal decay in preparations of *Vicia faba* leaf tissue containing normal and heavy water. Analogous results (not shown) were obtained with preparations of wheat leaf tissue and with D_2O containing chloroplasts. The bottom trace in fig.1 displays the low-frequency modulation caused by hyperfine interaction (hfi) and nuclear quadrupole interaction with chlorophyll nitrogens [13] and, in addition, a high-frequency modulation induced by hfi with protons, both of the molecules in which the unpaired electron is delocalized and of their nearest neighbours.

In the upper decay, along with the features mentioned, one observes additional minima in signal amplitude with a period of 440 ns, corresponding to the Zeeman frequency of deuterium in the applied external magnetic field $H = 3380$ Oe. This appears to be the first evidence for the presence of deuterium nuclei within a radius of a few ångstroms round the unpaired electron in this preparation. The experimental decay reported allows some quantitative information about the interaction of the unpaired electron with deuterium to be gained.

Since the contributions of different nuclei to the primary ESE modulation are multiplicative [14], the low-frequency modulation of the deuterium nuclei may be derived by dividing the upper envelope in fig.1 by the lower one. The result of the division, given in fig.2a, curve E, may be analytically expressed as $V(\tau) = V_D(\tau)/V_H(\tau)$, where $V_H(\tau)$ is the contribution to the lower modulation envelope of those protons which are substituted by deuterium in the sample giving the upper trace. For the envelopes in fig.1 the division of V_D by V_H results in high-frequency beats of small amplitude.

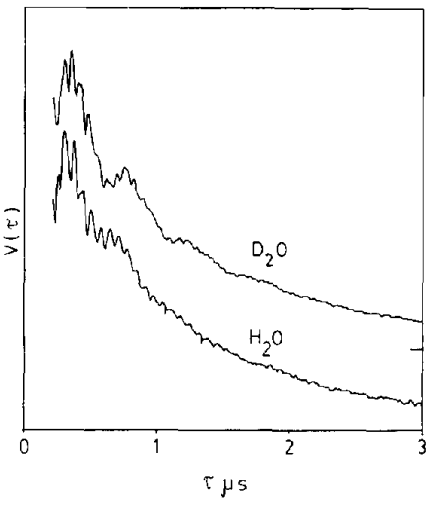

Fig.1. $P700^+$ primary ESE signal decay in preparations of freeze-dried leaf tissue of *V. faba* with H_2O and D_2O.

The frequency composition of the observed deuterium modulation is seen from fig.2b, presenting the cosine Fourier transform of curve E in fig.2a. The spectrum has two components, with maxima at 2.2 and 4.5 MHz, the amplitude of the latter being negative.

The spectral data may be interpreted in the following way. The frequency of 2.2 MHz coincides with the Zeeman frequency of the deuterium nuclei, ν_I, in the applied external magnetic field. ESE modulation with the same frequency is found in disordered systems with nuclei coupled with the unpaired electron by a weak anisotropic hfi. Such nuclei also cause modulation of low amplitude and negative sign [14] at a frequency close to twice the Zeeman frequency (i.e. 4.4 MHz) corresponding to a combination $\nu_\alpha + \nu_\beta$ of nuclear transition frequencies at two electron spin projections. For this kind of system estimates of the anisotropic hfi and the number of the deuterium nuclei contributing to the ESE modulation may be obtained from analysis of the modulation harmonic ν_I amplitude damping for times $\tau < 2\,\mu$s, where it is not yet affected by nuclear quadrupole interactions [15], according to the technique proposed in [16]. The amplitude of the harmonic with the Zeeman frequency, determined as $\lambda = 1 - V_{min}/V_{max}$ (V_{min} and V_{max} are the envelopes depicted by the dashed lines in fig.2), must decrease in the initial time region as

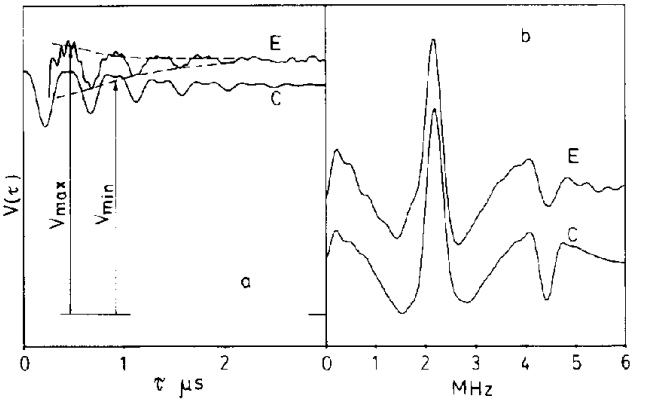

Fig.2. (a) Deuterium ESE modulation (E, experiment; C, calculation), (b) cosine Fourier spectra corresponding to the curves in panel a.

a linear function of τ^2. Analytically, this decrease is expressed as follows:

$$\lambda = \frac{32}{5} \sum_i \frac{T_{\Sigma i}^2}{\nu_I^2} \left(1 - \frac{4\pi}{14} T_{\perp i}^2 \tau^2\right). \quad (1)$$

Extrapolation of this linear dependence to zero time yields a value proportional to $\Sigma_i T_{\perp i}^2$, and the tangent of the slope to $\Sigma_i T_{\perp i}^4$ ($T_\perp$ is the perpendicular component of the deuterium nucleus anisotropic hfi tensor in the axial approximation).

The linear decrease of modulation amplitude as a function of τ^2 is observed with satisfactory precision for $\tau^2 < 1\ \mu s^2$ (fig.3). From the data given in fig.3, the initial value of modulation amplitude $\lambda_0 = 0.19$ and the slope $\Delta\lambda/\Delta\tau^2 = 0.1\ \mu s^{-2}$. Hence, $\Sigma_i T_{\perp i}^2 = 0.144\ MHz^2$ and $\Sigma_i T_{\perp i}^4 = 0.0268\ MHz^4$ may be found using eqn 1. Assuming N equivalent nuclei to contribute to modulation, we obtain $N = 0.77$ and $T_\perp = 0.43$ MHz.

Such a result seems to indicate that in the case under consideration, the contribution to the ESE modulation is made by a single nucleus coupled with the unpaired electron with the anisotropic hfi $T_\perp = 0.4$ MHz. The fact that the number of nuclei is slightly below unity may be related to the approximate character of the analysis [16] or to the partial substitution of protons in these centers by deuterons.

Numerical calculation of the modulation for one deuterium nucleus yields good agreement with the experimental amplitude and damping of the harmonic at $T_\perp = 0.4$ MHz. In calculations for the decrease of the harmonic $\nu_\alpha + \nu_\beta$ amplitude at large time τ, account has been taken of nuclear quadrupole interactions (nqi) [15,16]. The best agreement between experimental and calculated values for the axial nqi modulation patterns was obtained for values of quadrupole coupling constant $e^2Qq/h = 0.15$–0.2 MHz for different angles between axes of nqi and hfi tensors. For example, envelope C, shown in fig.2a, and a corresponding cosine Fourier spectrum (fig.2b, dead time $\tau = 200$ ns), were calculated for the following set of parameters: $T_\perp = 0.4$ MHz, $e^2Qq/h = 0.2$ MHz, asymmetry parameter of the electric field gradient at the deuterium nucleus $\eta = 0$, and the angle between nqi and hfi axes $\theta = 90°$.

Summing up the above, one may say that a comparative analysis of the $P700^+$ ESE modulation in the preparations containing normal and heavy water has allowed us to detect a deuterium

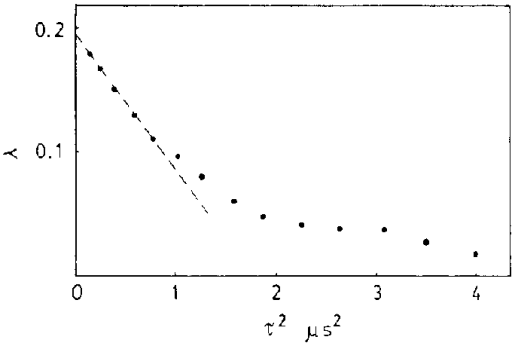

Fig.3. The dependence of modulation amplitude λ of harmonic ν_1 on τ^2.

resulting from isotopic substitution in the immediate surroundings of the P700 centers. The resulting value $T_{\perp} = 0.4$ MHz, in the point dipole approximation equal to $gg_1\beta\beta_1/r^3$, produces the distance $r = 3.1$ Å. However, taking into account that the unpaired electron in $P700^+$ is delocalised, this distance should be considered as an upper limit to the feasible values. Actually, the distance from the deuterium to the plane of the nearest chlorophyll molecule with spin density must be substantially lower.

As noted above, the experiments have been performed with preparations from three sources and in all cases we have obtained deuterium modulation envelopes with identical amplitude and kinetics of damping. This allows one to assume with some confidence that practically complete substitution of the exchangeable protons by deuterium takes place. The analysis of the modulation pattern resulted in the demonstration that a single deuterium nucleus contributes to the envelope observed and that it is situated in the immediate vicinity of P700.

Analogous experiments with isolated chlorophyll *a* oxidized with iodine in a solution of CH_2Cl_2 in CH_3OD or CH_3OH (1:3) have shown that the ESE signal decay of the monomeric chlorophyll radical cations practically lacks the deuterium modulation. Thus, the observation of the interaction with solvent protons needs a special structural organisation, absent in the solution of monomeric chlorophyll but present in the reaction centers of photosystem I. All these results allow the assumption that the deuterium manifesting itself in the $P700^+$ ESE signal decay belongs directly to a chlorophyll dimer aggregate. Whether it is in the water molecule which binds two parallel dimer macrocycles (the second water deuteron in this case could be unobservable if placed far from the regions of considerable electron spin density) or in some nucleophilic protein side group located in the immediate vicinity to primary donor chlorophyll molecules [8] remains to be established.

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