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Stark effect in P700, the primary electron donor of Photosystem I

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Quadratic Stark effect in CP1 pigment–protein complex was examined at low temperatures in the red spectral region. The Stark spectra of samples containing P700 in reduced form exhibit a strong negative band at 704 nm, which disappears on chemical oxidation of P700. The change in permanent dipole moment, $\Delta\mu$, of P700 on electronic excitation estimated from these spectra was found to be between 4.7 and 7.7 Debye units. It is suggested to reflect the charge-transfer contribution to the excited state of P700. For antenna chlorophyll, $\Delta\mu \cong 1$ D was obtained in accordance with the data for monomeric chlorophyll.

Photosynthesis; Photosystem I; Reaction center; Pigment–protein; P700; Stark spectrum

1. INTRODUCTION

The electronic structure of the excited state of the photosynthetic primary electron donor in Photosystem I of higher plants, P700, has been the subject of intense investigations. Among a variety of proposals that have been put forward to account for its specific absorption, circular dichroism and ESR spectra as well as for the redox properties [1,2], the hypothesis considering P700 to be a chlorophyll *a* dimer seems to be the best substantiated. In recent years, new experimental data concerning physical properties of P700 have become available. Resonance Raman spectroscopy of Photosystem I reaction centers has shown that the chemical as well as optical bleaching of P700 involves two molecules of chlorophyll *a* [3]. Photochemical hole burning experiments [4,5] pointed to a significant redistribution of electronic charge over the component molecules. Based on this, their authors postulated an essential role of the charge-transfer (CT) state in the lowest excited state of P700, in analogy with bacterial primary electron donors P870 and P960 for which similar phenomena were confirmed in the studies of the Stark effect [6–8]. On the other hand, the involvement of CT states in the electronic excitation of chlorophyll *a* dimer in vitro has been confirmed in our recent study of the Stark effect [9]. A significant increase in the permanent dipole moment on electronic excitation, $\Delta\mu = 5.2$ D was found for the (Chl · ethanol)$_2$ dimer, compared to $\Delta\mu = 1$ D for chlorophyll *a* monomer.

This paper presents a similar investigation on the Stark effect in chlorophyll–protein complex CP1, which is one of the preparations highly enriched in the reaction center of Photosystem I. It is shown that P700 exhibits a strongly enhanced Stark effect. The parameters of the Stark spectrum are used to estimate the change in the permanent dipole moment of P700 on electronic excitation.

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2. MATERIALS AND METHODS

The pigment–protein complexes were obtained by SDS dissociation of thylakoid membranes isolated from 14-day bean leaves, as described previously [10]. They were separated by electrophoresis in 0.7 mm slabs of polyacrylamide. All operations were performed at 4°C. The patterns containing CP1 were cut off and incubated for 75 min in darkness at 2°C in 70% glycerol containing either 1 mM sodium ascorbate or 0.5 mM potassium ferricyanide. After this treatment, two glass plates with transparent SnO_2 electrodes were applied to the slab, the sample was positioned in the cryostat and cooled to about 130 K.

The measuring setup used was that described previously [9]. To detect the quadratic Stark effect, the frequency of the applied electric field (960 Hz) was doubled and then used to drive the lock-in phase detector. The proportionality of the measured signals to the square of applied voltage was also checked experimentally. The transmitted DC and electric field-modulated AC light intensities were recorded simultaneously and numerically converted to absorption, A, and the Stark spectrum, ΔA. All measurements were performed using unpolarized light beam perpendicular to the sample. The methods of evaluation of relevant molecular parameters are described in section 3 and 4.

3. RESULTS

The absorption and Stark spectra of two samples differing in the redox states of P700 are shown in Figs. 1 and 2. The relative absorbance at about 700–705 nm ($\cong 14200$ cm^{-1}) was always slightly lower in oxidized samples than in reduced ones. If the absorption spectra of the reduced and oxidized samples are scaled so as to match each other at the maximum, their difference

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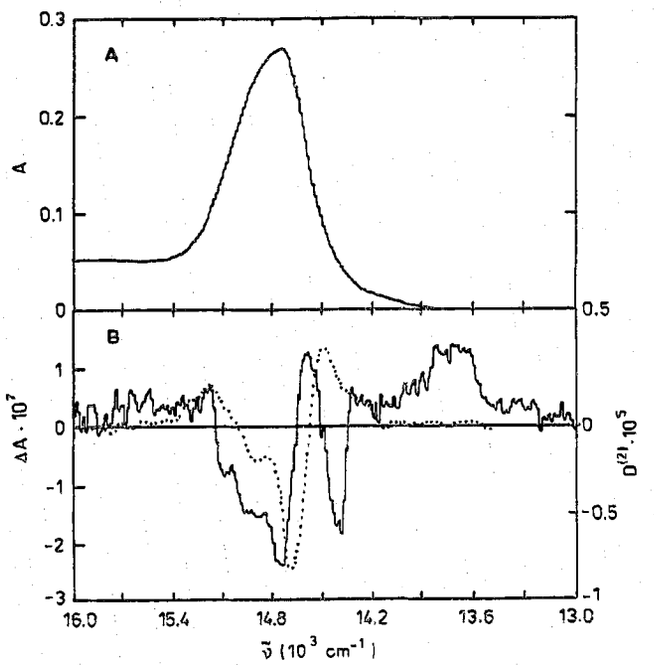

Fig. 1. (A) Absorption spectrum. (B) Stark spectrum (continuous line) and the second derivative of absorption (dotted line) for oxidized CP1 particles. Temperature: 127 K; electric field strength (RMS): 43570 V/cm.

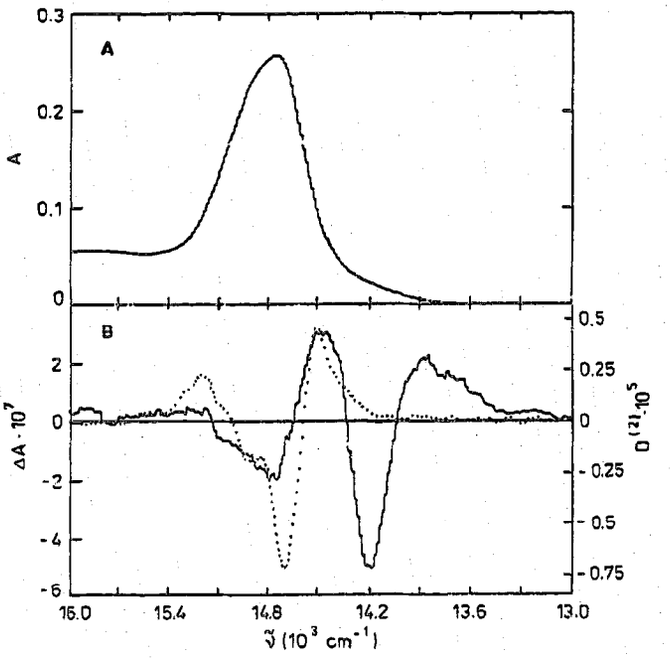

Fig. 2. Same as in Fig. 1 but for reduced CP1 particles. Temperature: 128 K; electric field strength (RMS): 35715 V/cm.

($A_{red}-A_{ox}$) at 700–705 nm is 1/40 to 1/30 of the maximum value of absorbance at 678 nm, consistently with the expected chemical bleaching of P700 in oxidizing environment.

These small differences in absorption are accompanied by much stronger effects in the Stark spectra. The Stark spectrum of oxidized CP1 (Fig. 1B) is split into two weak components. The broader one with minimum at 678 nm (14755 cm^{-1}) follows approximately the second derivative of the absorption spectrum but is slightly shifted to shorter wavelengths. The second component, centered at 693 nm (14410 cm^{-1}) and much narrower, is related to a weak inflexion in the second derivative at roughly the same wavelength. As the Stark effect in immobilized chlorophyll molecules is dominated by the change in the permanent dipole moment and thus the main contribution to it follows approximately the second derivative [9], it is inferred from the relative intensities of these bands in the Stark spectrum that the longer wavelength component is related to a chlorophyll species undergoing considerably larger change in the dipole moment on electronic excitation than most of the antenna chlorophylls.

The Stark spectrum of reduced CP1 (Fig. 2B) in the region corresponding to the absorption by antenna chlorophylls is quite similar to that of oxidized sample. The main difference is the presence of the strong negative band at 704 nm (14200 cm^{-1}) in the Stark spectra of reduced CP1, with positive shoulders on both sides. The intensity of this band is closely related to the treatment of CP1 with sodium ascorbate. In samples not subjected to any chemical treatment, this band is weaker and does not exceed the one related to antenna chlorophyll. We consider such dependence of this band on the chemical treatment together with its characteristic position at 704 nm to be the main arguments for ascribing this Stark band to P700.

4. DISCUSSION

For an isotropic sample in a rigid matrix, positioned perpendicularly to the light beam, the quadratic Stark effect related to the change in permanent dipole moment on electronic excitation in an electric field of intensity F, colinear with the light beam, is given by [6–9]:

$$\Delta A = \frac{(\Delta\mu)^2}{10\sqrt{2}\,h^2c^2} D^{(2)}(2-\cos^2\delta)\,F^2 \qquad (1)$$

where $\Delta\mu$ is the difference between the ground- and excited-state dipole moments, δ is the angle between the vector $\Delta\mu$ and the transition dipole moment, and $D^{(2)}$ denotes the weighted second derivative of the absorption spectrum: $D^{(2)} = \nu \cdot d^2(A/\nu)/d\nu^2$, with ν being the wavenumber.

The knowledge of the second derivative, $D^{(2)}$, is essential for a quantitative estimate of $\Delta\mu$ for P700 to be done. In the case of CP1, in which P700 occurs in relatively small proportion to antenna chlorophyll, about 1:45 [3,11], $D^{(2)}$ can be estimated indirectly by assuming a gaussian-like absorption band shape for P700: $A = G \cdot \exp\{-[(\nu-\nu_0)/w]^2\}$ (w is the 1/e halfwidth).

For such band, the width w estimated from the spacing of the zero-crossing points in the Stark spectrum, is 198 cm^{-1}. The amplitude G can be estimated in two ways: (i) by taking its value as equal to the difference between normalized spectra ($A_{red} - A_{ox}$) at 700–705 nm; or (ii) by assuming somewhat arbitrarily that the area under the gaussian corresponding to P700 is equal to 1/40 of the total area under the main absorption band of the reduced sample. Having determined the gaussian parameters and assuming the value of δ (cf. Eqn. 1) equal to 30° (a mean between 34°–40° for bacterial reaction centers [6–8], and 23° for the chlorophyll a dimer in vitro [9]), we obtain $(\Delta\mu)_{P700}$ = 6.9 D or 5.6 D. It should be noted that the assumed value of δ does not strongly affect these estimates, as for extreme values $\delta = 0°$ and $\delta = 90°$ the above values of $(\Delta\mu)_{P700}$ should be multiplied by 1.12 or 0.79, respectively. Generally, values of $(\Delta\mu)_{P700}$ obtained for different samples fall in the range 4.7–7.7 D for all possible values of δ and are evidently much higher than for monomeric chlorophyll a for which $\Delta\mu$ = 1 D [9]. This rather large value of $(\Delta\mu)_{P700}$ must be related to partial charge-transfer character of the lowest electronic transition in P700. It is also an indication that the structure of P700 is similar to the (Chl · ethanol)$_2$ dimer, at least with respect to the approximate C_2 symmetry and to the orbital overlap.

The Stark signals corresponding to antenna chlorophylls exhibit a remarkable deviation from the second derivative (Fig. 1B). At present, it is difficult to decide whether this deviation is due to an increase in molecular polarizability on electronic excitation or different values of $\Delta\mu$ should be ascribed to various spectral forms of antenna chlorophyll. The minimum value of ΔA in this region (at which the possible first-derivative term is virtually zero) inserted into Eqn. 1 leads to $\Delta\mu$ for antenna chlorophylls in the range between 0.8 and 1.1 D, very similar to the data for isolated chlorophyll molecules [9].

The local-field correction factor [6–8] is nearly the same (especially at low temperatures) for the samples investigated here as well as for chlorophyll a monomer and dimer in vitro and for bacterial reaction centers. Thus, we prefer to compare the 'crude' values estimated for various samples. The approximate values of $(\Delta\mu)_{P700}$, 4.7–7.7 D, are similar to $\Delta\mu$ found for the dimer (Chl · ethanol)$_2$ in vitro (5.2 D [9]), and comparable to $\Delta\mu$ found for bacterial primary donors P870 (6.5–9.6 D [6–8]) and P960 (6.5–8.2 D [7,8]). Thus, the results obtained in this study evidently support the dimer hypothesis for the structure of P700.

The nature of a positive feature at ≅730 nm (13750 cm^{-1}) in Stark spectra of oxidized samples (Fig. 1B) and the assignment of the Stark band at 693 nm, as well as a close examination of the Stark spectra of P700 and of antenna chlorophylls remain to be the subject of further studies.

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