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High-resolution triplet-minus-singlet absorbance difference spectrum of photosystem II particles

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The triplet state of the primary donor of photosystem II particles prepared from a mutant of *Chlamydomonas reinhardtii* has been studied at 1.2 K with absorbance-detected ESR in zero-magnetic field (ADMR). Two sets of resonances with slightly different zero-field splitting parameters $|D|$ and $|E|$ were observed, $|D| = 285.5$, $|E| = 38.8$ and $|D| = 288.8 \times 10^{-4}$ cm^{-1}, $|E| = 42.2 \times 10^{-4}$ cm^{-1}, respectively. Both sets of $|D|$ and $|E|$ values are close to those found for P^T-700, as are the sublevel decay rates $k_x = 930 \pm 40$, $k_y = 1088 \pm 50$ and $k_z = 110 \pm 5$ s^{-1}. The AMDR-detected triplet-minus-singlet absorbance difference spectrum of P^T-680 is very similar to that of P^T-700 and closely resembles that of covalently connected Chl *a* dimers in vitro. We conclude that P-680 is a Chl *a* dimer whose general structure is similar to that of P-700.

Triplet state *Photosystem II* *ODMR* *Electron spin resonance* *Absorbance difference spectrum*

1. INTRODUCTION

The triplet state of the primary donor of photosystem II, P^T-680, was first observed in [1,2] with high-field ESR. Its zero-field splitting parameters were determined and it was demonstrated that its yield titrates with the intermediary acceptor $Pheo^-$ with a midpoint potential of $E_m = -604$ mV [3]. This and its polarization pattern indicated that the triplet state is formed by the back reaction P^+-680 $Pheo^- \longrightarrow P^T$-680 Pheo, and as such it is analogous to the triplet states of the primary donor in bacterial photosynthesis and of photosystem I (reviews [4,5]). Here, we report a zero-field ESR study of P^T-680 in photosystem II particles. We have determined accurate values of the zero-field splitting parameters $|D|$ and $|E|$, and of the sublevel decay rates k_x, k_y and k_z. Furthermore, we have recorded with high-resolution the low-temperature triplet-minus-singlet absorbance difference spectrum, employing the new technique of absorbance-detected magnetic resonance (ADMR) [6,7].

In the particles studied we observed two sets of resonances with slightly different values of $|D|$ and $|E|$, which are close to those previously observed with high-field ESR [1]. They are slightly different from the zero-field parameters of P^T-700 in photosystem I particles [8]. The amplitude of the corresponding resonances is about equal in magnitude. The two sets are attributed to two types of P-680 which differ in geometrical properties. These two types may correspond to a heterogeneity in reaction centers, as the so-called α- and β-centers believed to be present in chloroplasts [9,10], or they are a result of slight changes brought about by the isolation procedure. The decay rates $k_{x,y,z}$ are close to those recently determined for P^T-700 in PS I particles [8,11,12].

The triplet-minus-singlet absorbance difference (T − S) spectrum of P-680 is quite similar to that of P-700 [8]. The latter spectrum closely agrees with the T − S spectrum of dimeric chl *a* in methylcyclohexane [13], and deviates considerably from the T − S spectrum of monomeric chl *a* in vitro [14]. This was taken as a strong indication that P-700 is a dimeric chl *a* complex [7]. By analogy we tentatively conclude that P-680 also is a dimeric chl *a* complex.

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2. MATERIALS AND METHODS

Photosystem II particles were prepared from a mutant of *Chlamydomonas reinhardtii* as in [15]. The samples were slowly frozen in liquid nitrogen in the presence of 10 mM sodium ascorbate. Absorbance-detected magnetic resonance (ADMR) of the triplet state at 1.2 K was done as in [6,7]. Decay rates were measured with the pulse method [16]. Triplet-minus-singlet absorbance difference spectra were recorded using the ADMR technique as in [7,8].

3. RESULTS AND DISCUSSION

3.1. *Zero-field splitting parameters and decay rates*

The ADMR transitions of P^T-680 monitored at 682.5 nm are displayed in fig.1. The linewidth and lineshape of the low-frequency transition is similar to that of P^T-700 [8]. The high-frequency transition, however, is clearly split. The two sets of $|D|$- and $|E|$-values corresponding to the observed resonances are collected in table 1, together with those of P^T-700. It is seen that they are quite similar, yet the differences are distinct and outside the limits of error. The zero-field splitting parameters are close to those measured for monomeric chl *a* in vitro in various solvents (table 1) [17]. Note that the values of $|D|$ and $|E|$ for chl *a* in vitro are solvent-dependent, and show a range that comprises the values for P^T-680 and P^T-700.

The two triplet states of P-680 that have slightly different zero-field parameters may correspond to two somewhat different types of PS II reaction centers, analogous to the α- and β-centers reported for chloroplasts [9,10]. We cannot exclude, however, that one of these reaction center types is produced by the isolation procedure. We have found earlier that PS I particles from different bat-

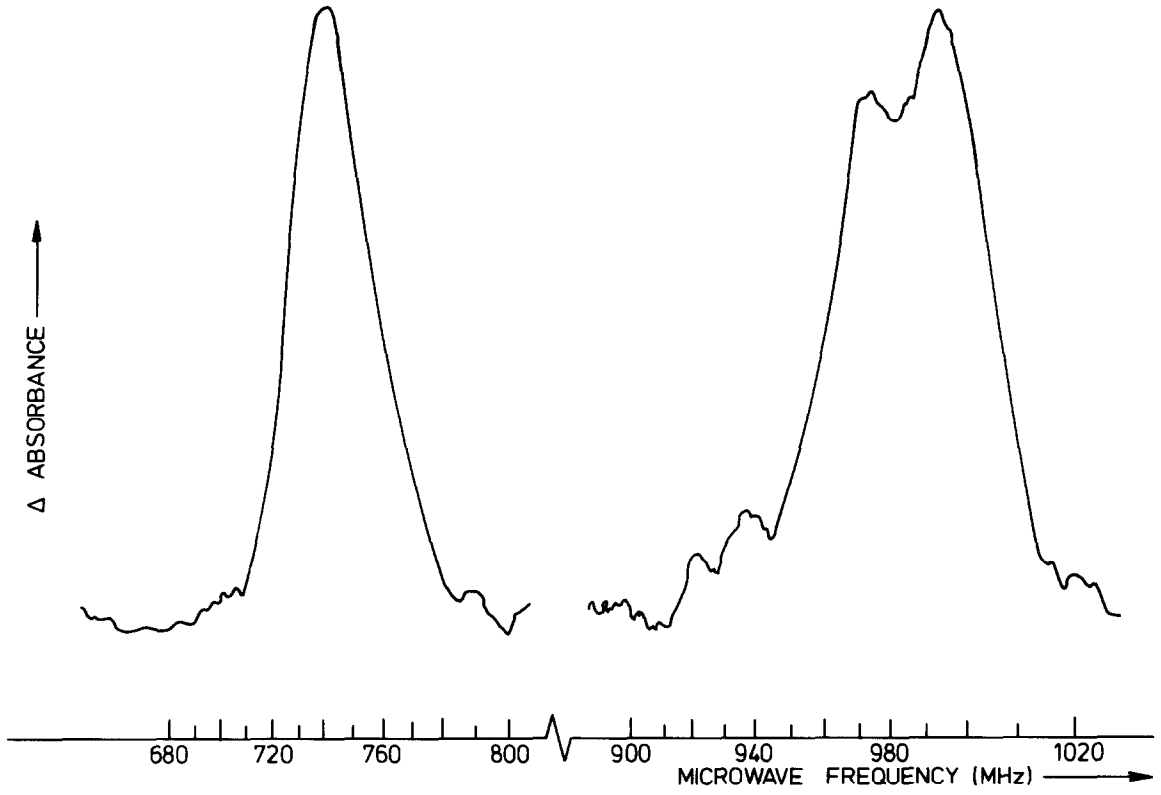

Fig.1. ADMR transitions of photosystem II particles at 1.2 K. The detection monochromator was set at 682 nm with 3.2 nm resolution. The spectra are single scans with amplitude modulation of the microwaves at 35 Hz and lock-in detection. Total scan time for one transition 100 s, response time 1 s.

Table 1

Zero-field splitting parameters in $cm^{-1} \times 10^4$ of P^T-680, P^T-700 and ^{3}Chl *a*

	$\|D\|$	$\|E\|$	Detection λ (nm)	Ref.
P^T-680	285.5 ± 0.7	38.8 ± 0.7	682.5	Here
	288.8 ± 0.7	42.2 ± 0.7	682.5	Here
P^T-700	281.7 ± 0.7	38.3 ± 0.7	697	[8]
Chl *a*·H_2O in *n*-octane	305 ± 3	38 ± 3		[17]
Chl *a*·MTHF in MTHF	291 ± 3	59 ± 3		[22]
Chl *a* in *n*-octane	280	38		[20]

ches or isolated following a different procedure vary somewhat in their values of the zero-field splitting parameters [8].

The precise resonance frequencies of the zero-field transitions are somewhat dependent on the wavelength at which the absorbance is monitored (table 2). This effect was previously encountered for the triplet state of the bacterial primary donor [18], and was attributed to site effects. A site is defined as a group of molecules or complexes that have identical optical and magnetic parameters which can be distinguished from those of other sites. Sites are generated by pigment–host interactions, such as seen in chl *a* dissolved in *n*-alkanes [19], or by the formation of pigment complexes of different configuration [18]. We have argued that in the bacterial photosystem, site effects probably arise from small differences in the geometry of the bacteriochlorophyll dimer making up the primary donor [18]. We note, that the resonance frequencies of table 2 decrease with increasing wavelength at which the triplet resonances are monitored, and that there is a smooth transition from the values of P^T-680 to those of P^T-700. By analogy to the bacterial primary donor, we take this as evidence that the absorption bands of P-680 and P-700 are composed of many narrow, overlapping 0–0 transitions. The exact transition wavelength is then governed by either the relative position of two or more molecules making up P-680, or by interactions of the P-680 pigment with the protein matrix

Table 2

Variation of transition frequency with detection wavelength

	Detection wavelength (nm)	$\|D\| - \|E\|$ transition (MHz ± 2)	$\|D\| + \|E\|$ transition (MHz ± 2)
PS II	675.0	754	990
	677.5	747	980 (shoulder), 997
	680.0	743	975 (shoulder), 995
	682.5	740	973, 993
	685.0	737	980 (shoulder), 990
	687.5	735	980
	690.0	731	970
	692.5	730	965
PS I	697.5	730	960
	702.5	720	953

[18]. We will show below that the T – S spectrum points to the former possibility. This suggests that the peak wavelengths of the bleaching of P-680 and P-700, viz. 682.5 and 697.5 nm, respectively, reflect the maximum of a distribution with a width of 15 to 20 nm and that the general structure of P-680 is not essentially different from that of P-700.

The triplet-sublevel decay rates (table 3) are close to those found for P^T-700. They are qualitatively similar to reported values of the decay rates of ^{3}Chl *a* in vitro [20–22]. Note, however, that some of the latter values have been measured by fluorescence-detected magnetic resonance employing the so-called 'equilibrium method', which technique can give only approximate results which may differ considerably from the exact rates as measured by the pulse method [23].

The triplet state parameters (viz., the $|D|$- and $|E|$-values) and the decay rates $k_{x,y,z}$, do not allow us to discriminate between P-680 being a monomeric chl *a* or a chl *a* dimer. Variation of ligands to the central magnesium of chl *a* may change the values of $|D|$ and $|E|$ by 10% (table 1), whereas admixture of small charge transfer

Table 3

Triplet sublevel decay rates in s^{-1} of P^T-680, P^T-700 and chl *a*

	k_x	k_y	k_z	Ref.
P^T-680	930 ± 40	1088 ± 50	110 ± 5	Here
P^T-700	990 ± 100	1010 ± 100	92 ± 5	[8]
Chl *a*				
MTHF	1180 ± 120	830 ± 80	180 ± 30	[22]
MTHF	620 ± 80	1120 ± 20	140 ± 40	[21]
n-Octane	661 ± 89	1255 ± 91	241 ± 15	[20]

contributions to the triplet wave function of a dimeric complex may also strongly vary the triplet parameters [24]. Hence, the close correspondence between the triplet parameters of P^T-680 and of monomeric chl *a* in vitro cannot be taken as evidence that P-680 is monomeric or that it is a parallel dimer. In contrast, the T – S spectrum shows features strongly suggesting that P-680 is in fact a dimeric chl *a* complex.

3.2. *The triplet-minus-singlet absorbance difference spectrum*

In fig.2a the T – S spectrum of P^T-680 – P-680 is displayed together with that of P^T-700 – P-700 [8]. It is seen that the general aspect of the two spectra is very similar. Both spectra exhibit a strong bleaching at 682.5 and 697.5 nm, respectively, a pronounced positive peak at 665 and 672 nm, respectively, and a structured bleaching in the Soret region. When comparing the spectra with published T – S spectra of monomeric chl *a* in ethanol [14] and of a covalently-linked chl *a* dimer in methylcyclohexane [13] (fig.2b) it is seen that in the dimer, but not in the monomer, in vitro spectrum next to the bleaching in the red, a pronounced positive band appears. This positive band was attributed to an appearing monomer absorption band, the triplet state being localized on 1 of the 2 chl *a* molecules on an optical time scale [13]. By analogy, we attribute the positive bands at 665 nm (P-680) and 672 nm (P-700) to appearing monomer bands of a dimeric chl *a* complex in which excitonic interaction is broken by triplet formation. As, also in the shorter wavelength region, the T – S spectrum of P-680 is much closer to the T – S spectrum of dimeric chl *a* in vitro than to that of monomeric chl *a*, we infer that P-680 is a dimeric chl *a* complex.

Aside from the precise wavelengths at which the shifts, the bleachings and the appearing bands occur, the only major difference between the T – S spectra of P-680 and P-700 is the presence of a small band at 685 nm in the P-700 spectrum. This band was attributed to a shift of an accessory chl *a* pigment, possibly one of the chl *a* acceptors [8]. In the P-680 spectrum the band is missing. This may be caused by the positive band at 665 nm and the bleaching at 682.5 nm being some 10 nm closer together than the corresponding features in the P-700 spectrum, thereby possibly obscuring a small shift in this region. Alternatively, the Pheo *a* acceptor or chl *a* molecules adjacent to P-700 are less affected by the formation of P^T-680, or the shift has disappeared because of the isolation procedure for the PS II particles. We noted in [8] that the 685 nm feature in PS I particles is rather sensitive to ageing, and sometimes is present only as a weak shoulder.

Another feature which appears to be present in the T – S spectrum of P-700 but not in that of P-680, is a weak band around 820 nm. The origin of this band in the T – S spectra of P-700 is unclear. It is also seen in the P^+-700 – P-700 and in the P^+-680 – P-680 absorbance difference spectra [25,26], and weak bands around 800 nm are observed in the absorbance difference spectra of chl a^+ and chl a^- in vitro [27,28], but not in that of ^{3}Chl *a* [29]. Possibly the 820 nm band is due to a charge transfer contribution, which then is weaker in the triplet of the PS II particles.

The T – S spectrum of P-680 may be compared with its oxidized–reduced absorbance difference spectrum [30–32]. The two types of difference

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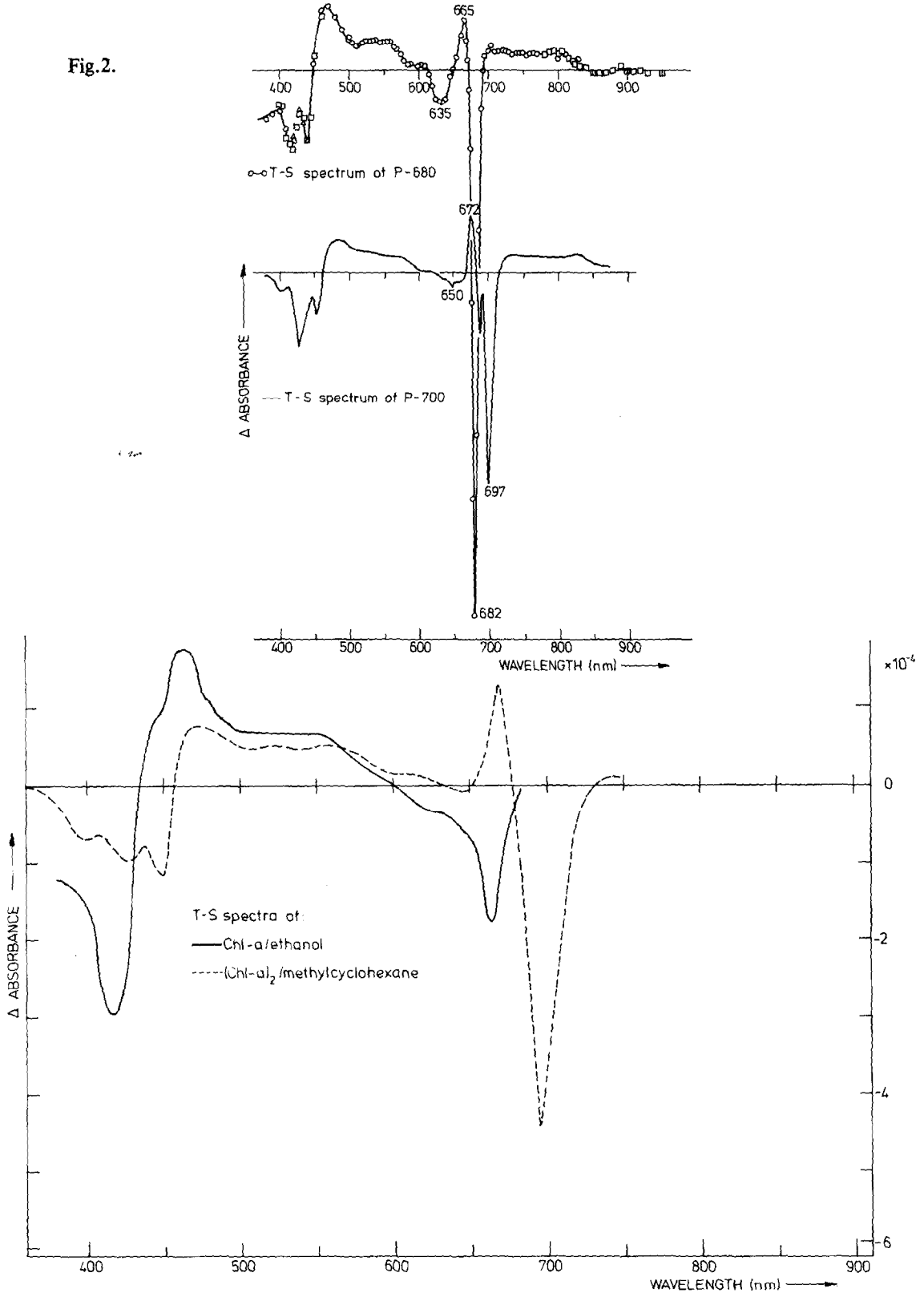
Fig.2. (a) The ADMR-monitored P^T-680 minus P-680 absorbance difference spectrum of PS II particles (T – S spectrum) at 1.2 K. (—○—), (—□—) and (—△—): data taken on the same sample at different runs. The resonance microwaves were set at 990 MHz, the optical resolution was 3.2 nm. A spectrum monitored at 970 MHz was identical to the reproduced spectrum. Drawn line: the T – S spectrum of P-700 recorded with the ADMR method [8]. (b) The flash-induced T – S spectrum of monomeric chl *a* in ethanol at ~25°C [14] (——), and that of a chl *a* dimer in methylcyclohexane at −78°C [13] (– – –).

Fig.2.


665
635
672
650
697
682
400
500
600
700
800
900
o–o T-S spectrum of P-680
T-S spectrum of P-700
Δ ABSORBANCE
WAVELENGTH (nm)
×10⁻⁴
0
-2
-4
-6
T-S spectra of:
Chl-a/ethanol
(Chl-a)₂/methylcyclohexane

spectra share the bleaching phenomena, they may differ in the appearance of bands due to 'monomer' absorption, in the triplet–triplet and cation absorption bands, and in induced band shifts. In the P^+-680 – P-680 spectrum, the bleaching at 680 nm is about twice as wide as in the T – S spectrum, and no positive band around 665 nm is present, although the spectrum peaks around 660 nm. Both differences may be at least partly caused by the difference in temperature at which the spectra were recorded (300 K, P^+-680 – P-680; 1.5 K, P^T-680 – P-680). It is well known that for PS I particles lowering the temperature causes a pronounced band sharpening, and a sharp increase of a positive band at 690 nm [33,34]. In addition, the narrower aspect of the T – S spectrum monitored via the ADMR technique may be partly due to band sharpening as a result of site-selection by the magnetic resonance frequency [18]. In order to sort out these two contributions (temperature and site-selection) we will have to wait till a low-temperature P^+-680 – P-680 spectrum becomes available.

The oxidized–reduced spectrum of P-680 was interpreted in [35] to be composed of the bleaching of a dimer chl *a* band, and the joint appearance of absorption bands of a monomer chl *a* and a monomeric chl *a* cation. This interpretation corresponds to our interpretation of the T – S spectrum of P-680 as being due to a triplet state localized on one of a pair of chl *a* molecules, but both interpretations run counter to the suggestion in [27] that P-680 is a monomeric chl *a* species. Their argument is mainly based on redox-potential considerations. In view of the fact that very little is known about the influence of complex formation, and of protein–pigment interactions on the redox midpoint potential of chlorophylls, we prefer to be guided by the new experimental evidence of the T – S spectrum which suggests that P-680 is composed of a pair of chl *a* molecules, that strongly interact in the singlet state, and only weakly in the triplet state.

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