Structural organization of Photosystem I reaction centers

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Using a phosphoroscopic attachment to the dichrograph, light-induced circular dichroism spectra have been measured for chlorophyll–protein complexes of Photosystem I. Minor components at 672, 678 and 685 nm are observed in these spectra in addition to the components of dimer splitting of the P700 Q_y transition at 691 and 698 nm. The minor components are due to the Chl_{672}, Chl_{678} and Chl_{685} forms of antenna chlorophylls, the optical activity of which is changed 2–4% as a result of P700 oxidation. It is suggested that P700 is not an isolated dimer but that it is included in a local complex comprising 8–10 chlorophyll molecules with an exciton level splitting value of 120–140 cm^{-1}.

Photosynthesis *Chlorophyll–protein complex* *Photosystem I* *Circular dichroism* *P700*

1. INTRODUCTION

Photooxidation of the primary electron donor P700 of Photosystem I of green plants and algae is accompanied by symmetric dimeric splitting of the Q_y (691 and 698 nm) and B_{xy} (434 and 445 nm) transitions in light-induced CD spectra [1,2]. This phenomenon has been ascribed to the disappearance of resonance interaction between the chlorophyll molecules in the oxidized P700 dimer (Chl-Chl^+) [1]. Recent EPR measurements of $P700^+$ in deuterated and ^{13}C-enriched algae have suggested that only one molecule in $P700^+$ is oxidized [3]. The absorption band of the chlorophyll that is not oxidized in $P700^+$ appears at 688–689 nm in the difference spectra as a result of a 1.5–2 nm spectral shift and an increase in the dipole strength of the short wavelength splitting component at 691 nm [2].

However, it is not clear why such a long wavelength position of the non-oxidized Chl molecule band is apparent in the spectrum of $P700^+$, as disappearance of the resonance interaction in a photooxidized dimer should result in a shift to a shorter wavelength region [4]. It is also difficult to explain the multicomponent structure of the low temperature difference spectra of P700 [5–7].

Here we have studied the minor components in the light-induced P700 CD spectra in the region of the Q_y transition. The results indicate that these components are due to the disappearance of the resonance interaction of the oxidized Chl molecule in $P700^+$ with the antenna chlorophyll forms.

Abbreviations: CD, circular dichroism; Chl, chlorophyll; PSI, Photosystem I; CPI, chlorophyll–protein complex of PSI; P700, primary electron donor of PSI

2. MATERIALS AND METHODS

Three different types of CPI complexes were isolated from *Pisum sativum* chloroplasts. CPI(a) was separated by preparative electrophoresis with SDS (detergent/Chl = 10, w/w) as in [8]. Two of them, CPI(b) and CPI(c), were fractionated by hydroxylapatite chromatography from membranes solubilized with Triton X-100 (detergent/Chl = 76, w/w) as in [9]. However, in variance with [9], the material adsorbed on the column was washed by 10 mM Tris–HCl (pH 8) containing 0.05% Triton

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until the eluate was almost colourless. The CPI(b) fraction was eluted from the column with 0.01 M phosphate while the CPI(c) fraction was eluted with 0.2 M phosphate after further washing with 1% Triton.

Analytical electrophoresis in slab gels was performed as in [10]. It revealed that CPI(a) contained only two polypeptides of 65–70 kDa; CPI(b), in addition to these two polypeptides, possessed three low-molecular-mass polypeptides of 8, 15, 20 kDa; CPI(c) contained, in addition to all the abovementioned polypeptides, an admixture of 25 and 45 kDa polypeptides.

CD spectra were measured with a JASCO-40AS dichrograph equipped with a data processor. Low-temperature measurements were made in a 2 mm cell with addition of 60% glycerol. Light-induced CD spectra (dark minus light) were measured using a specially designed phosphoroscopic attachment to the dichrograph. A sample in a rectangular 1 cm cell was illuminated by white light of 4×10^4 erg·cm^{-2}·s^{-1} through two fibre-optic light guides at a 90° angle to the measuring beam. Light-induced CD changes at 20°C were registered using a signal accumulation technique (spectra were measured point-to-point 1–2 nm, measurement time for each point was 3–5 min). The noise level was less than 3×10^{-6} units of a difference in absorbance $\Delta A = A_l - A_r$. CD spectra are presented as ΔA and as difference in extinction coefficients $\Delta\epsilon = \epsilon_l - \epsilon_r$ ($M^{-1} \cdot cm^{-1}$). An extinction coefficient of 64 $mM^{-1} \cdot cm^{-1}$ was used to calculate the amount of P700 [11]. Light-induced absorption changes were measured as in [12]. Protein concentration was determined according to [13] and protein secondary structure was calculated from CD spectra as in [14,15].

3. RESULTS

The 3 complexes differ from each other in the quantity of antenna chlorophylls. The Chl/P700 ratio for CPI(a) is 50–60, for CPI(b) 65–70, and for CPI(c) 40–45. However, the light-induced absorption spectra of P700 are similar for all complexes and resemble those reported earlier [2,5,6,16]. Fig.1 shows that light-induced CD spectra of P700 are similar for all CPI. As well as the main bands of dimer splitting at 691(+) and 698(−) nm, they contain minor components at

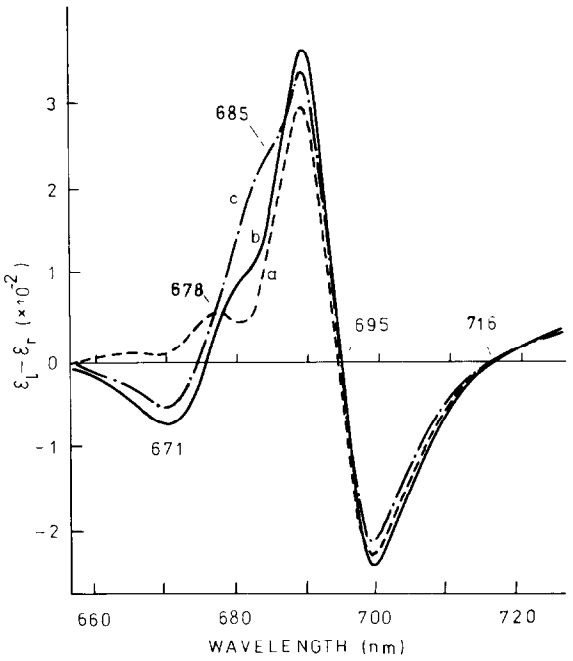

Fig.1. Light-induced CD spectra (dark minus light) of P700 of CPIs (a–c) differing in Chl/P700 ratio (see text for details): 0.05 M Tris–HCl (pH 8.0), 10 mM sodium ascorbate, 10 nM methyl viologen, 20°C.

672(−), 678(+) and 685(+) nm with bandwidths of 12–13 nm. The exact positions and bandwidths of all bands at 20°C were found from the Gaussian deconvolution of light-induced CD spectra [17].

The positions of the minor bands in the P700 light-induced CD spectra are almost identical to those of the main bands of antenna chlorophyll (Chl_{672}, Chl_{678}, Chl_{685} and Chl_{693}). These forms predominate in the low-temperature absorption and CD spectra of all CPI (fig.2). Comparison of figs 1 and 2 also indicates that a similarity exists between the relative intensities of the minor components in P700 light-induced CD spectra and amplitudes of the corresponding bands in the CD spectrum of antenna Chl: the band at 672 nm is very weak in spectra of CPI(a) and the band at 685 nm is the strongest in CPI(b).

For CPI(c), the amplitude of light-induced CD changes at 700 nm makes up 10–12% of the ΔA values of the antenna Chl_{693} form at 693 nm. The ratio is constant and does not change when the CPI is destroyed in the presence of high concentration of detergents or urea or at high temperatures.

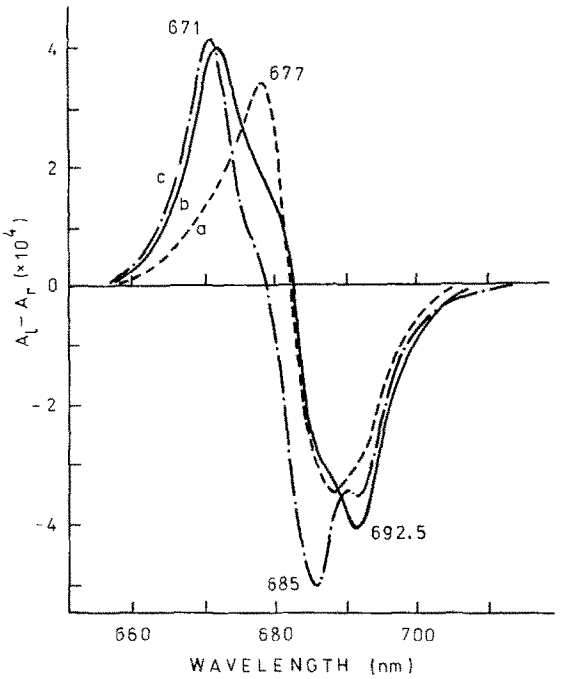

Fig.2. CD spectra of antenna chlorophyll forms of CPIs differing in Chl/P700 ratio; absorbance at 677 nm = 0.7, −196°C.

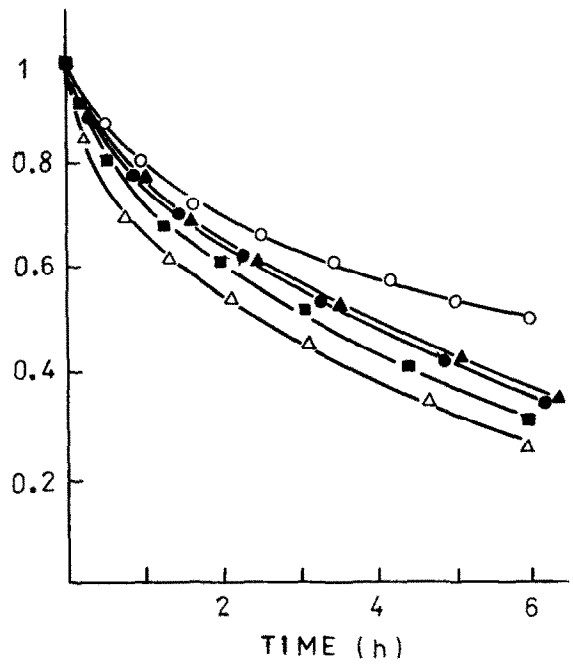

Fig.3. Kinetics of P700 inhibition and destruction of the antenna chlorophyll of CPI(c) by Triton X-100 (detergent/Chl = 450). Measurement conditions were the same as in fig.1. (○—○) Absorbance at 680–710 nm; (●—●) $(A_l - A_r)$ at 693 nm; (▲—▲) light-induced absorption changes at 700 nm; (■—■) light-induced CD changes at 700 nm and (△—△) at 690 nm.

Thus, fig.3 shows that the destruction by 1% Triton (detergent/Chl = 450) of the long wavelength, optically active chlorophyll forms occur simultaneously with P700 inhibition. CD spectra of CPI(c) and light-induced CD spectra of P700 alter insignificantly during the initial steps of the incubation. The simultaneously occurring destruction of all the antenna Chl forms, and the P700 inhibition, point to the cooperation of the two processes. An especially close correlation (correlative coefficient, 0.95–0.97) is observed between the changes in the ΔA value of the 693 nm (ΔA_{693}) in the CD spectrum of CPI and light-induced absorption changes of P700 at 700 nm (ΔD_{700}). From this, the P700 concentration can be calculated by the following equation: $\Delta D_{700} = 38(\pm 5)(A_l - A_r)_{693}$.

It is likely that the Chl_{693} form is an obligatory component of the PSI reaction centre and the inhibition of P700 by the detergent is due to its destruction. This conclusion is supported by the fact that for P700 itself there is a difference between the kinetics of disappearance of the light-induced CD and absorption signals (fig.3). As a result, the value of $\Delta\epsilon$ for P700 decreases from 255 to 220 $M^{-1} \cdot cm^{-1}$ at 700 nm and from 390 to 310 $M^{-1} \cdot cm^{-1}$ at 690 nm. This indicates that the value of $\Delta\epsilon$ (and probably the value of rotational strength) is not a constant molecular characteristic of the dimeric interaction in P700.

The stability of the antenna Chl forms against low detergent concentration is likely to be due to conformational stability of the protein structure.

Table 1

The protein secondary structure of CPI, isolated with different detergent/Chl ratios

SDS/ Chl	α-Helix	β-Anti-parallel	β-Paral-lel	β-Turn	Aperiodic
10	0.31	0.02	0.13	0.14	0.39
15	0.36	0.04	0.12	0.20	0.28
20	0.35	0.03	0.14	0.18	0.30

CD spectra of CPI(a) and CPI(b) are similar to each other in the 190–250 nm region. The protein secondary structure of CPI is characteristic for the parallel α/β protein domains [18]. The electrophoretic control showed that the strong CD spectra dependence in the 200–250 nm region, on addition of SDS described earlier [19], is mostly due to a decrease in light-scattering as a result of reassociation of protein complexes.

4. DISCUSSION

Both the similarity of the protein secondary structure of CPIs isolated using different detergent/Chl ratios, and the similarity between Chl CD spectra for isolated CPI and CPI in the membrane [20] point to the native state of the isolated CPI. The spectral Chl forms (Chl_{663}^{673}, Chl_{672}^{676}, Chl_{678}^{682}, Chl_{693}^{697}) which predominate in the absorption and CD spectra of CPI were also found from the absorption (663, 672, 685) and fluorescence (682 and 697) spectra in highly purified PSI particles with a Chl/P700 ratio of 8:10 [21]. Thus, 6–8 molecules are enough to form the above-mentioned antenna chlorophylls. One may suppose that CPI(c), with a Chl/P700 ratio of about 40, contains 3–4 'local complexes' composed of 8–10 molecules. Their interaction leads to an exciton level splitting of 6–7 nm (120–140 cm^{-1}).

The existence of the minor components in light-induced CD spectra of P700 can be explained if we assume that P700 is incorporated in such a 'local complex' and that its photooxidation leads to the disappearance of the resonance interaction between the oxidized Chl in $P700^+$ and the nearest antenna Chl molecules. As a result, there are some changes in the rotational and dipole strengths in the whole system of interacting oscillators. This explains the minor bands observed at 672, 678 and 685 nm in low temperature P700 difference absorption spectra and also the long wavelength position of the absorption band (689 nm) of the non-oxidized molecule in P700.

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