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CD spectroscopy provides evidence for excitonic interactions involving red-shifted chlorophyll forms in photosystem I

E. Engelmann[a], T. Tagliabue[a], N.V. Karapetyan[b], F.M. Garlaschi[a], G. Zucchelli[a], R.C. Jennings[a,*]

[a] *Centro C.N.R. Biologia Cellulare e Molecolare delle Piante, Dipartimento di Biologia, Università di Milano, Via Celoria 26, 20133 Milan, Italy*
[b] *A.N. Bakh Institute of Biochemistry, Russian Academy of Sciences, Leninsky Prospect, 33, 117071 Moscow, Russia*

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Abstract Selective destruction of the strongly dichroic red-shifted chlorophyll form (C709 nm) in photosystem I (PSI) trimers from *Spirulina*, by either non-selective high intensity illumination (photobleaching) or incubation with low concentrations of Triton X-100 is accompanied by changes in the circular dichroism spectrum of the same amplitude and of opposite sign at 677 nm. The data are interpreted in terms of a dimeric chlorophyll structure with excitonic bands at these two wavelengths. Similar photobleaching experiments with PSI-200 from maize also suggest the presence of bulk antenna/red form excitonic interactions.

Key words: CD spectroscopy; Excitonic interaction; Photobleaching; Red chlorophyll form

1. Introduction

The antenna systems of plants and cyanobacteria contain a large number of chlorophyll (Chl) molecules, the lowest electronic transition (Q_y) of which absorbs at higher energies than that of the primary electron donor. This is important for fast and hence efficient energy flow to the primary electron donor, as relatively slow thermal activation processes are avoided. It is now well established that energy transfer rates between most Chl antenna sites occur in a subpicosecond to a few picosecond time frame [1–6]. An intriguing exception to this situation is provided by the so-called red antenna Chl forms of photosystem I (PSI). These are a small number of molecules which absorb at lower energies than the primary electron donor [7–17] and which, in the most extreme case, are slightly over 4 kT below the purely electronic transition of P700 [14,17,18]. It is now fairly well established that these red forms do not have a role in focussing energy on the primary electron donor [19,20,21] as was initially thought [8,22], and in fact they impose an energy diffusion limitation on the trapping rate in higher plant PSI [23]. Interestingly, it has recently been demonstrated that they may play an important role in light harvesting within a system of dense vegetation where their property of absorbing just outside the main absorption band becomes important [24].

It is often suggested that the large bathochromic shifts of the red forms come about as the result of strong excitonic interactions between, or with, antenna molecules belonging to the so-called bulk antenna i.e. with average Q_y absorption maxima around 675–680 nm (e.g. [16,25]), though direct supportive evidence is scarce. Consistent with this suggestion is their unusually large reorganisation energy due to strong electron–phonon coupling [12,18,26], the large linear pressure shifting rate of the red absorption state and large permanent dipole [26] demonstrated for the 718 nm red form in *Synechocystis*. In addition, in their hole burning measurements, with burn wavelengths above 702 nm, Ratsep et al. [26] demonstrated the presence of broad difference spectrum holes at 692 and 699 nm which could be excitonically correlated with the 718 nm form, though this point was not rigorously demonstrated. Savikhin et al. [27], reporting on pump–probe anisotropy decay experiments with *Synechocystis* demonstrated that for 710 nm excitation the initial anisotropy at 680 nm was approximately −0.5. This suggested the presence of mutually, orthogonally polarised exciton states near these wavelengths and is the most direct evidence to date for excitonic states involving red forms.

We have recently demonstrated that the major red form in *Spirulina* trimers, absorbing at 709 nm, is selectively bleached when samples are non-selectively illuminated [18]. This band is strongly dichroic and it was noticed that changes in the CD signal between 670 and 680 nm accompanied photobleaching of the 709 nm CD band. In this communication we present evidence, from CD difference spectroscopy of photobleached and detergent treated samples, that the 709 nm band is excitonically correlated with a state absorbing near 677 nm, thus suggesting a dimeric organisation for these Chls. Similar photobleaching experiments are also presented for the strongly dichroic red tail of higher plant PSI-200.

2. Materials and methods

Membranes from cells of the filamentous cyanobacterium *Spirulina platensis* were isolated using a French press and double centrifugation at 100 000 × *g* (1 h at 4°C) to remove phycobilisomes as described [14]. Membranes were treated with dodecyl β-D-maltoside (detergent/Chl ≈ 15), and PSI trimeric complexes isolated using column chromatography on DEAE-Toyopearl according to Shubin et al. [14]. The Chl/P700 ratio in complexes was about 90. PSI complexes were sus-

*Corresponding author. Fax: (39)-2-2660-4399.
E-mail: robert.jennings@unimi.it

Abbreviations: PSII, photosystem II; PSI, photosystem I; Chl, chlorophyll

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pended in 50 mM Tris–HCl buffer (pH 8.0) and stored at 77 K. Before using, samples, after thawing, were dissolved in 50 mM Tris–HCl buffer (pH 8.0).

PSI-200, which contains the entire antenna complement, was prepared from maize plants using octyl β-D-glucopyranoside as previously described [11] and samples for measurements were prepared as described in the same reference.

Absorption spectra were measured with an OMA III (EG and G, Model 1469) as described previously [28].

Circular dichroism spectra were measured in a Jasco J-600 spectropolarimeter equipped with a red extended photocathode (Hamamatsu R2228), bandwidth was 2 nm. Samples were routinely placed at 2 cm from the photomultiplier and the optical density was 0.6 at the Q_y absorption maximum.

Photobleaching of *S. platensis* trimers and PSI-200 was performed using white light (34 000 E m^{-2} s^{-1}) between 20 and 90 min at 4°C.

3. Results and discussion

3.1. Spirulina trimers

Fig. 1 shows the absorption spectra of *Spirulina* trimers in the Q_y region at room temperature (RT) and 80 K together with the CD spectrum at RT. Whilst the red tail of the absorption spectrum is fairly featureless at RT a clear CD structure near 709 nm is evident, which seems to correspond to an 80 K absorption structure evident near this wavelength, as previously reported [18].

It was previously demonstrated that the 709 nm absorption and CD band is subject to photobleaching when non-selectively illuminated [18]. In Fig. 2A this is shown for the CD spectrum for different photobleaching times, after normalisation to the negative lobe minimum. It is apparent that progressive bleaching of the negative 709 nm band is paralleled by a decrease in the positive lobe, near its maximum at 670 nm. This is shown more clearly in Fig. 2B where the difference spectra for two photobleaching times are presented. The negative difference spectrum lobe is at 709 ± 1.3 nm while that of the positive lobe is at 677 ± 1.4 nm. The amplitudes of the positive and negative difference spectra lobes are precisely correlated during photobleaching (Fig. 3). As it is unlikely, though not impossible, that two pigments display almost identical sensitivity to photobleaching, these data are most simply interpreted in terms of a Chl dimer with excitonic bands at 709 nm and 677 nm.

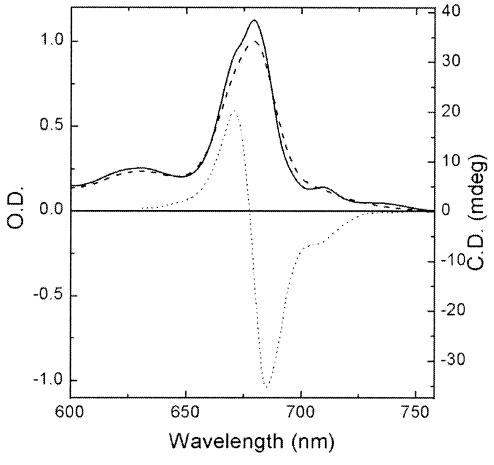

Fig. 1. Absorption spectra of *Spirulina* PSI trimers measured at 280 K (dashed line) and 80 K (continuous line) and the CD spectrum measured at 280 K (dotted line).

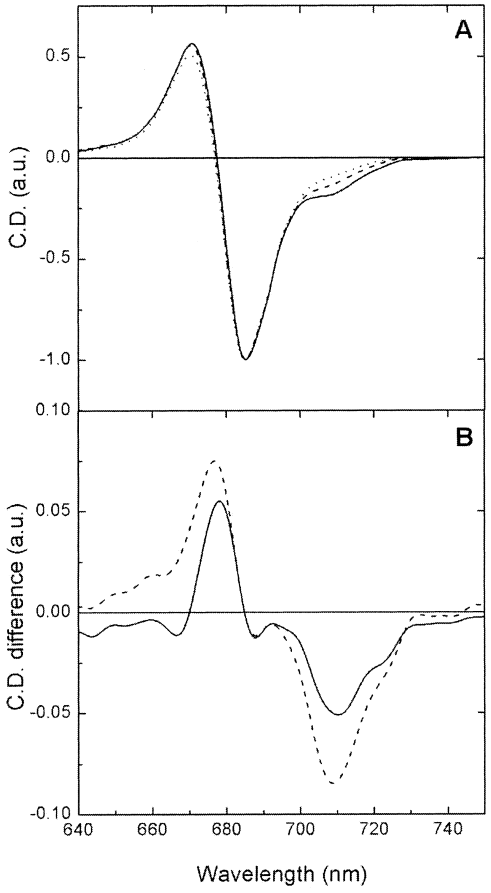

Fig. 2. The effect of photobleaching on the CD spectrum of *Spirulina* trimers. A: control (solid line), photobleached for 10 min (dashed line) and 20 min (dotted line). The spectra were normalised to the minimum of the negative lobe. B: CD difference spectra due to photobleaching as in A. The spectra were calculated as control minus photobleached.

We have noticed in earlier studies that the red Chl forms are particularly sensitive to incubation with detergent [12]. *Spirulina* trimers were therefore incubated with very low concentrations of Triton X-100 and the difference spectrum determined (Triton X-100 minus control, Fig. 4). This difference spectrum structure closely resembles that due to photobleaching (Fig. 2B) with the negative lobe maximum at 709 nm and the positive lobe at 677 nm. In Fig. 3 we have also plotted the amplitudes of the positive and negative difference spectra lobes for different Triton X-100 concentrations. It is seen that these points lie close to those for the photobleaching difference spectra. These data therefore strongly support the idea that the 709 nm transition is in fact the low energy excitonic band of a Chl dimer, with the high energy transition located at 677 nm. This conclusion is in good agreement with Savikhin et al. [27] who suggested the presence of excitonically correlated states near 710 nm and 680 nm in *Synechocystis*.

In addition to the main positive difference spectrum lobe near 677 nm, a minor structure is clearly present in the detergent experiment around 660 nm. This structure also seems to be present in the photobleaching difference spectra, though less pronounced. We are at present unable to provide an interpretation of this, though it may reflect structural rearrangement accompanying the loss of the 677/709 nm dimer.

It will be noticed that while the amplitudes of the positive

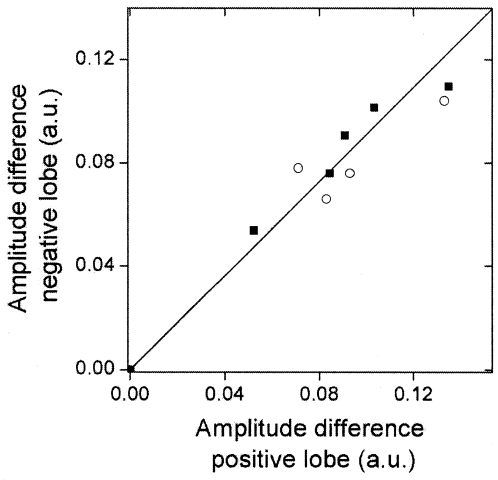

Fig. 3. The amplitude of the CD negative difference spectrum lobe versus the amplitude of the positive difference spectrum lobe of *Spirulina* PSI trimers, for different times of photobleaching (squares; 10–50 min) and after incubation with different Triton X-100 concentrations (circles; 0.032–0.1% v/v). Difference spectra were calculated as control minus phototobleached or detergent treated. The straight line represents the linear regression for the photobleaching experiment.

and negative difference spectra lobes are very similar, the areas subtended by the lobes are somewhat different due to the fact that the bandwidths are different, with the 677 nm positive lobe being about 10–11 nm (FWHM) and the positive lobe at 709 nm being about 17–18 nm wide. This of course means that the spectra are non-conservative, which is not in agreement with conventional excitonic theory. However it should be pointed out that in conventional excitonic theory only the zero phonon line (purely electronic transition) is considered, whereas at RT the phonon bands dominate the absorption structure.

The energy separation between the low and high energy excitonic bands is 666 cm^{-1}. If the interacting monomers were isoenergetic, without interaction they would absorb at 693 nm and the interaction energy (J) would be half of the energy separation i.e. 333 cm^{-1}. As it is rather unlikely that the two monomer site energies are equal, due to protein effects, this value must be taken as an upper limit.

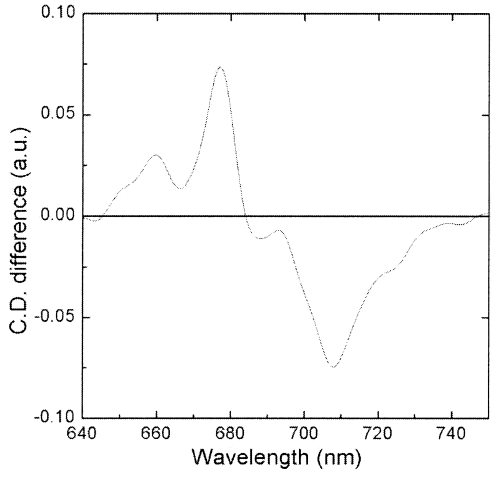

Fig. 4. The effect of incubation of PSI trimers of *Spirulina* with Triton X-100(0.032% v/v). Data are presented as the difference spectrum (control minus Triton X-treated) after normalisation to the negative lobe minimum.

3.2. PSI-200

The CD spectrum for the Q_y region of PSI-200 is presented in Fig. 5A together with its absorption spectrum. While the positive lobe ($\lambda_{max} \approx 670$ nm) is similar to that of *Spirulina*, the negative region is more structured, with features present near 684, 691, 706 and 735 nm, as indicated by the arrows.

Photobleaching experiments, of a similar kind to those described above for *Spirulina* trimers, were performed (Fig. 5A). It is apparent that the strongly dichroic red tail is markedly sensitive to photobleaching, and this, as with *Spirulina*, is paralleled by changes in the positive lobe of similar intensity. This is more clearly shown in the difference spectra of Fig. 5B, where the bleaching in the red tail is maximal between 700 nm and 710 nm. Obviously the situation in PSI-200 is more complex than in *Spirulina*, due to the presence of a number of dichroic red forms in the former, all of which seem to be photobleached. However the general impression is that the CD amplitude changes in the red tail of PSI-200 are closely paralleled by the changes near 670 nm which are of opposite sign. Though it is difficult to comment on the band width of the red tail, due to the bleaching of overlapping bands, it

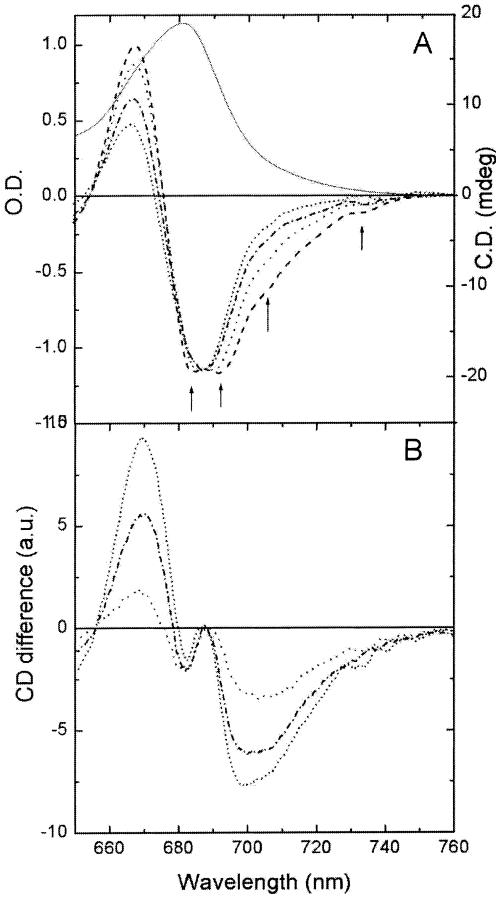

Fig. 5. A: The absorption and CD spectra of PSI-200 from maize and the effect of photobleaching on CD. CD spectra have been normalised to the negative lobe. The arrows indicate the approximate positions of the spectral features on the CD spectrum of unbleached PSI-200. B: The CD difference spectra (control minus photobleached) of PSI-200. Photobleaching time was: 0 min, solid line; 30 min, dashed line, 60 min, dotted line; 90 min, dot-dashed line.

would seem that the difference spectrum associated with the main bleaching (700–710 nm) is considerably broader than the accompanying 670 nm bleaching, as with *Spirulina*. Thus while the data on PSI-200 do not permit such a straightforward interpretation as the *Spirulina* case, they seem to indicate pigment–pigment interactions giving rise to excitonic bands in the bulk are which associated with those in the red tail.

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