S31-033

The major red form in *Spirulina* PSI trimers (C709) is the low energy band of an excitonic dimer

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Keywords: photosystem I, CD spectroscopy, red spectral forms, excitonic interaction

Introduction

The low energy chlorophyll *a* spectral forms of photosystem I (red forms) have received a lot of attention both from the point of view of their biological function and their physical characteristics. It is now fairly widely accepted that they probably bring about an antenna-based kinetic limitation to energy trapping (*eg* Fischer and Hoff, 1992; Croce et al 2000). Their primary function seems to be that of light harvesting in dense vegetation systems (*eg* Rivadossi et al, 1999). It is often suggested that the large red shifts of the red forms may be brought about by strong Coulombic interactions with other chloropyll *a* molecules leading to excitonic splitting, with the red form representing the low energy excitonic band of chlorophyll *a* dimers or higher order aggregates (*eg* Gobets et al, 1994; Ratsep et al, 2000). However little direct evidence for this is available except for the pump-probe anistropy experiments of Savikhin et al (1999) which suggested the presence of excitonic bands near 710nm and 680nm. One of the methods of choice for investigating excitonic coupling between pigments is circular dichroism (CD). In the simplest case of strongly interacting and identical monomers coupling between the purely electronic transitions gives rise to CD bands of opposite sign and equal intensity (*eg* Cantor and Schimmel, 1980), while non interacting pigments have usually only a weak CD signal which coincides with the absorption band. That the red forms are often strongly dichroic is well established (eg Koehne et al, 1999; Cometta et al, 2000), however it has not been possible to date to identify the high energy CD band and hence unambiguously demonstrate the excitonic nature. Recently we demonstrated that in PSI trimers of *Spirulina* the major red form, which absorbs near 709nm, is photooxidised upon exposure to high light fluences, with sufficient selectivity to permit its spectroscopic "isolation" at room temperature (Cometta et al, 2000). The CD band at 709nm disappears in parallel with the absorption band at this wavelength. In the present study we have used this to examine whether photooxidation induces changes in the CD signal in the high energy chlorophyll pool (bulk antenna) which are both correlated in intensity and opposite in sign to those of the 709nm band.

Materials and methods

Trimers of *Spirulina platensis* were isolated and spectroscopic manipulations performed as previously described (Cometta et al, 2000). Circular dichroism spectra were measured at both

room temperature and 120K in a Jasco spectropolarimeter (mod. J-600) equipped with a red extended photocathode (Hamamatsu R2228) with an OD of 0.6 at the Q_y absorption maximum. Samples were placed 2cm from the phototube.

Photobleaching was performed using white light (34,000μEm^{-2}s^{-1}) for 20-90 minutes at 275K. Incubation with Triton X-100 was performed using low concentrations in the range 0.032-0.1% (w/v).

Results and Discussion

The absorption and CD spectra measured at room temperature and 120K are presented in Figure 1. While the absorption spectrum is structureless above 700nm the CD spectrum displays distinct structure which gaussian decomposition describes by a single band with maximum near 709nm at room temperature and two bands near 709nm and 730nm at 120K.

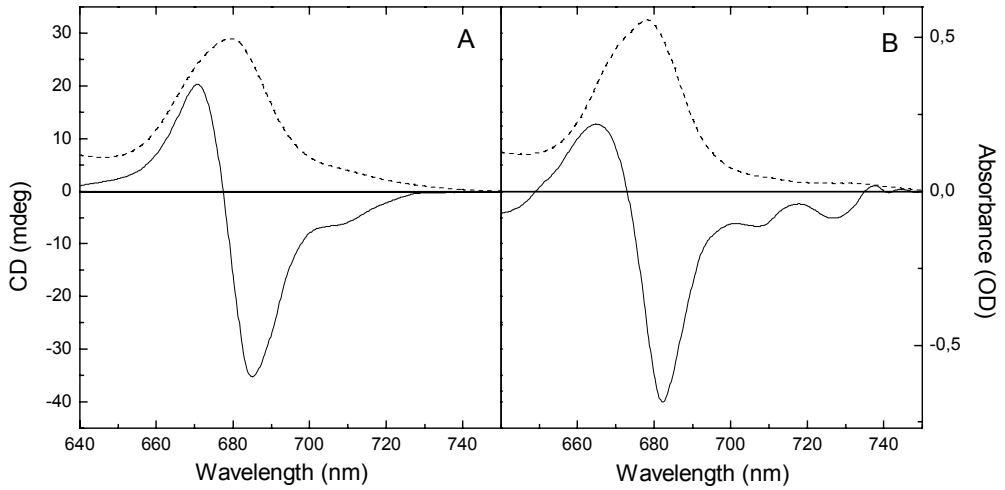

Fig. 1. Absorption and circular dichroism spectra of *Spirulina* trimers at 280K (A) and 120K (B) for the Qy region.

In Figure 2A the effect of photooxidation on the room temperature CD spectrum, after normalisation to the main negative band, is shown. The signal associated with the 709nm red form is clearly diminished and changes are also evident in the positive lobe near 670nm. The

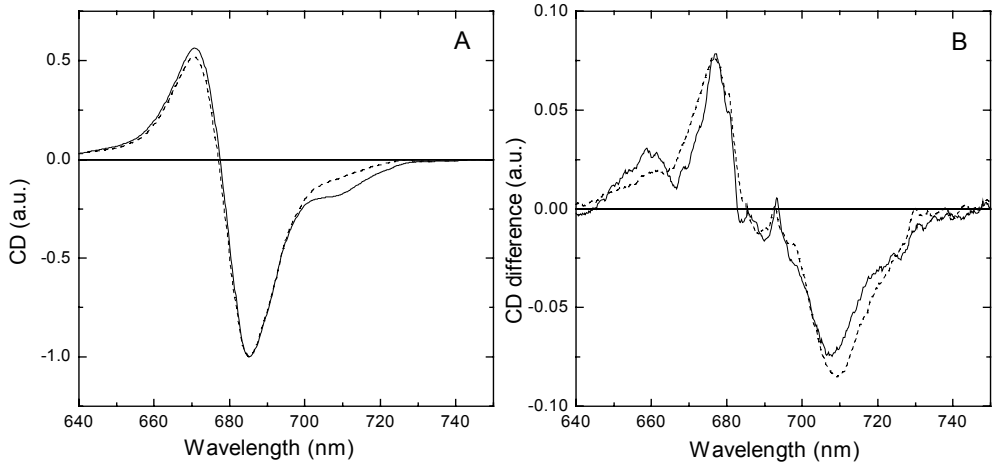

Fig.2 The effect of photobleaching (20mins) on the CD spectrum of *Spirulina* trimers (A). Normalisation was to the main negative lobe. (B) difference spectra (control minus photobleached sample, dashed line) and (control minus Triton X-100; 0.035%, full line).

difference spectrum presented in Figure 2B clearly shows the photooxidation induced changes with the difference spectrum shape having the characteristic form of an excitonic dimer spectrum. The negative lobe has its minimum near 709nm and the positive lobe its maximum near 677nm. Also shown in Figure 2B is the CD difference spectrum for PSI trimers treated with a low concentration of the detergent Triton X-100, a treatment know to disrupt red forms (Koenhe et al, 1999). Also in this case disruption of the 709nm band is accompanied by a CD change of opposite sign near 677nm. In addition a minor structure of positive sign is evident near 660nm.

In order to establish whether there is in fact a quantitative correlation between the positive and negative difference spectra lobes, their respective amplitudes were measured after different times of photooxidation and also upon incubation with different detergent concentrations. The data are presented in Figure 3 which shows that the difference spectra positive lobe amplitudes scale linearly with those of the negative lobe.

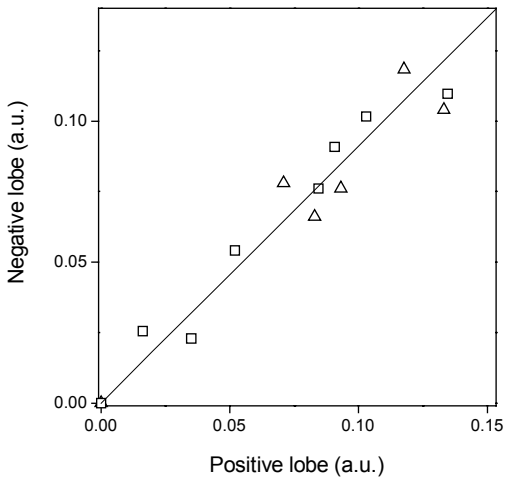

Fig. 3. Correlation between the amplitudes of the positive and negative CD difference spectra lobes for samples which had been either photobleached for different times (squares) or incubated with different concentrations of Triton X-100 (triangles).

The data presented in Figures 1-3 strongly suggests that the 709nm red form is excitonically correlated with a band in the "bulk" chlorophyll region which absorbs near 677nm. The energy separation between the high and low energy excitonic bands is thus about 660cm^{-1}. In the simple assumption, which certainly need not be correct, that the monomer energies are equal and that the entire energy difference is due to excitonic splitting, the Coulombic interaction energy (J) would thus be one half of this value ie 330cm^{-1}. This would imply a value for $\kappa/R^3 = J\cdot(5.05\mu_a\mu_b)^{-1} = 2.6$ (where κ is the transition dipole orientation, R is the Mg-Mg distance and μ_a and μ_b are the transition moment dipole vectors which are taken as equal to 5 Debye), which is certainly not unreasonably large for the PSI antenna crystallograpic structures available (Fromme et al, 1996).

The appearance of a strongly dichroic state at 730nm at 120K and which is absent at room temperature can be easily explained if one assumes that also the 730 state is the low energy excitonic transition of a dimer. The absence of a CD band at this wavelength would then be due to an "in plane" orientation of the transition dipoles with tilting out of plane occuring due to low temperature induced volume changes in the sample.

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