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Rapid Report

Excitation energy quenching in aggregates of the LHC II chlorophyll-protein complex: a laser-induced optoacoustic study

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The aggregation of isolated LHC II leads to the formation of an efficient excitation energy quenching mechanism which has been proposed as a model for ΔpH-dependent quenching in vivo. Here, I use laser-induced optoacoustic spectroscopy to show that the associated heat release is complete within 360 ns. The implications for the photochemical mechanism are discussed.

The structural and spectroscopic properties of LHC II, the major light-harvesting antenna complex of Photosystem II in green plants, have recently been the subject of intensive study [1–3]. Most studies have used the isolated complex in one of two states:

a. Detergent-solubilised LHC II trimers.

b. An aggregate form induced by the presence of Mg^{2+} with little or no detergent present.

It is not yet clear which form of LHC II most resembles the native configuration of LHC II in the thylakoid membrane. The two forms of isolated LHC II differ strikingly in their spectroscopic characteristics. While the solubilised LHC II trimers display the long fluorescence lifetime expected for an efficient light-harvesting complex, a powerful quenching mechanism is present in the aggregated state, leading to a much shorter fluorescence lifetime and a lower steady-state fluorescence yield [4–6]. The photochemical mechanism of the quenching process has not yet been determined. However, it is of particular interest since it has been proposed that a similar mechanism may been involved in photoprotective energy dissipation associated with ΔpH-dependent quenching in vivo [5,7].

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Abbreviations: Chl, chlorophyll; E_0, laser pulse energy; H, amplitude of the initial pressure wave in the LIOAS signal; H_n, energy-normalised optoacoustic signal ($H_n = H/E_0$); LHC II, light-harvesting Chl *a*/*b*-binding protein of Photosystem II; LIOAS, laser-induced optoacoustic spectroscopy.

Previous measurements of energy quenching in aggregated LHC II have used steady-state [5] or time-resolved [4,6] fluorescence spectroscopy. I have used laser-induced optoacoustic spectroscopy to give a direct measure of the additional heat release in the aggregated state and to investigate the timescale of the heat release.

LHC II was isolated as an Mg^{2+}-induced aggregate from dark-adapted spinach leaves by the method of Burke et al. [8]. The LHC II preparation had a Chl *a*/*b* ratio of 1.6 (determined according to the extinction coefficients of Porra et al. [9] after extraction in 80% acetone). The LHC II was resuspended in a buffer containing 100 mM sorbitol, 50 mM Tricine (pH 7.0) and briefly sonicated to break up any large particles of the aggregate. Ethylene glycol was added to 33% to improve the acoustic coupling of the medium. The final chlorophyll concentration was 15 μM and the absorbance at 650 nm was 0.454 cm^{-1}. The disaggregated form of LHC II was generated by the addition of 0.4 % n-octyl β-D-glucopyranoside and 0.4% digitonin. It was found necessary to add additional detergent to counteract a tendency to aggregate in the presence of ethylene glycol. Fig. 1 shows 77 K fluorescence emission spectra for the aggregated and disaggregated samples. The aggregated sample shows a characteristic quenching of the main emission peak at about 680 nm, with relatively high emission from a long-wavelength peak at 700 nm [5,6]. The disaggregated sample shows a small shoulder at 700 nm which may indicate some residual aggregation due to the presence of ethylene glycol. The room-temperature fluorescence yield of the

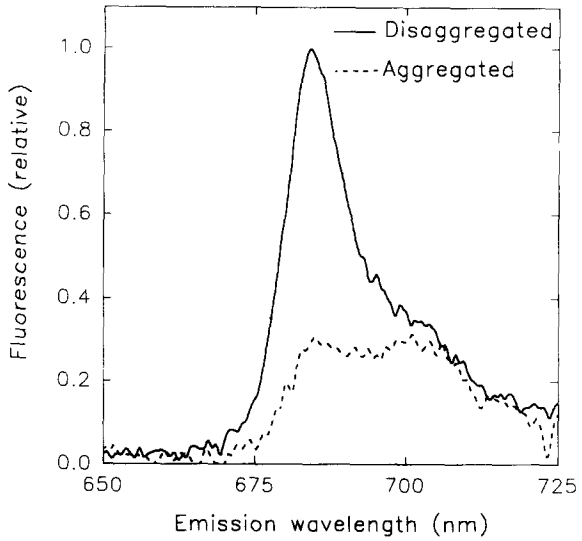

Fig. 1. Fluorescence emission spectra for aggregated and disaggregated LHC II at 77 K. A few drops of the sample suspension were adsorbed onto filter paper and frozen in liquid nitrogen. 77 K spectra were recorded as in [5], with excitation at 475 nm.

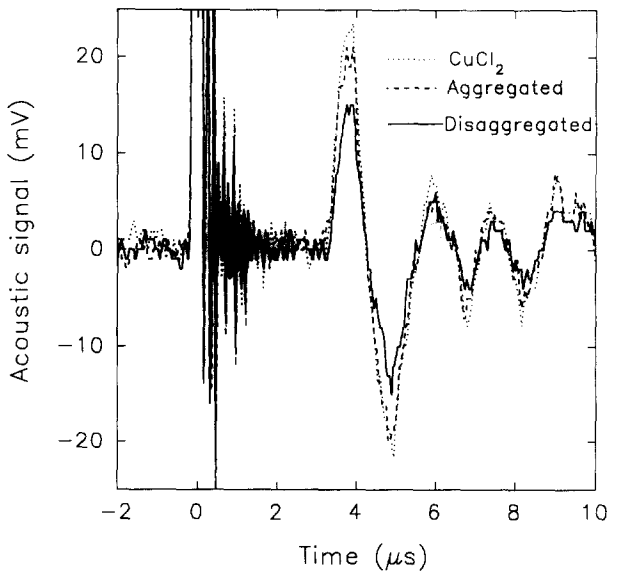

Fig. 2. LIOAS signals from aggregated and disaggregated LHC II and a $CuCl_2$ solution as a calorimetric standard. The laser pulse energy was 180 nJ.

disaggregated sample was higher than that of the aggregated sample by a factor of about 3.7 (Table I).

In LIOAS, the sample is excited by a short pulse of laser light and the pressure wave resulting from heat release by the sample is detected by piezoelectric transducers [10]. The development of resonant ceramic transducers has allowed the detection of heat release with high sensivity down to submicrosecond timescales [11]. The time-resolution of the technique is determined by the acoustic transit time across the diameter of the laser beam [11]. The speed of sound in 33% ethylene glycol was experimentally determined to be about 1400 m/s, and I used a laser beam contracted to 0.5 mm diameter with two biconvex lenses. Under these conditions, the amplitude of the initial pressure wave detected by the piezoelectric transducers is proportional to the integrated heat release during the first 360 ns following the laser pulse. 10 ns laser pulses were generated at a frequency of 1.25 Hz by an excimer pumped dye laser (EMG 50E and FL1002, Lambda-Physik, Göttingen, Germany). The dye laser was tuned to 650 nm with DCM as laser dye. Pulse energy was measured with an Ealing laser power meter and decreased with combinations of Balzers neutral density filters. The laser pulse passed through the centre of a 3 ml, 1 cm square glass flow cuvette (Hellma, Müllheim, Germany). The LIOAS signal was detected by two 4 mm thick 400 kHz resonant piezoelectric transducers (constructed by the workshops of the Max-Planck-Institut für Strahlenchemie, Mülheim an der Ruhr, Germany with PZT ceramic, Vernitron [11]) attached to opposite walls of the cuvette. The signals were added, further amplified (Analog Modules 351) and stored with a computer-linked 20 MHz transient recorder (R2000 from Rapid Systems, Seattle, OR, USA). The measurement was triggered by a reflection of the laser pulse detected by a photomultiplier. The signals from 100 pulses were averaged. The sample was pumped at about 7.5 ml/min through the flow cuvette to prevent photodamage due to excessive exposure to the laser pulse.

Fig. 2 shows LIOAS signals from aggregated and disaggregated LHC II and a $CuCl_2$ solution in the same buffer mixture with the same absorbance at 650 nm (±1%) as the sample. These signals were obtained at a pulse energy of 180 nJ. Assuming 14 Chl per LHC II monomer [12], this corresponds to about 0.8 absorbed photons per LHC II trimer during the course of the 10 ns pulse. $CuCl_2$ releases all absorbed energy as heat within the timescale of the measurement, and thus acts as a calorimetric standard [11]. The initial

TABLE I

Fluorescence and heat emission from aggregated and disaggregated LHC II

The ratio F_{680}/F_{700} is taken from the data in Fig. 1. Room temperature fluorescence yields were measured with a modulated fluorescence measurement system (Hansatech, King's Lynn, UK). Heat emission is the fraction of absorbed energy released as heat within the first 360 ns after the laser pulse. It is calculated by dividing the optoacostic signal (H_n) from the sample by the signal from the $CuCl_2$ calorimetric standard. The average H_n at the three lowest pulse energies was used (Fig. 3).

LHC II:	F_{680}/F_{700} (77 K)	Fluorescence (relative)	Heat emission	Energy not released as heat
Disaggregated	2.97	1.0	0.70	0.30
Aggregated	0.91	0.27	0.88	0.12

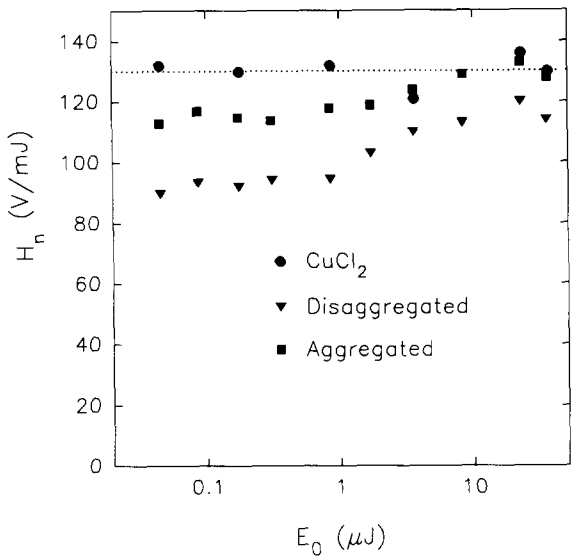

Fig. 3. Dependence of the energy-normalised LIOAS signal (H_n) on laser pulse energy (E_0). $H_n = H/E_0$, where H is the amplitude of the signal due to the initial pressure wave (Fig. 2).

pressure wave due to heat release from the sample can be seen about 4 μs after the laser pulse, which is accompanied by a burst of electrical noise (Fig. 2). The latter part of the signal is due to the ringing of the resonant transducers and acoustic reflections within the cuvette [11].

Fig. 3 shows the dependence of the energy-normalised LIOAS signal (H_n) on pulse energy, over a range of pulse energies from 45 nJ (about 0.2 photons/trimer) to 36 μJ (about 160 photons/trimer). The $CuCl_2$ standard shows a constant H_n, since the heat release is simply proportional to the input pulse energy. At low pulse energies, the LHC II samples show H_n which is lower than the $CuCl_2$ standard and approximately constant. The lower heat release from the LHC II samples indicates competing forms of energy conversion. One of these will be energy loss due to fluorescence; energy storage in the long-lived triplet excited states of chlorophylls and carotenoids is also a possibility. Note, however, that most absorbed energy is released as heat (Table I), in contrast to photosynthetically active samples where most absorbed energy is stored in the form of long-lived charge-separated states [11]. At high pulse energies, H_n from the LHC II samples increases (Fig. 3), presumably due to exciton annihilation, which will compete with fluorescence [13].

At low pulse energies, the heat release from aggregated LHC II is about 26% higher than that from disaggregated LHC II (Fig. 3 and Table I). The energy not released as heat is therefore decreased by a factor of 2.5 upon aggregation (Table I). This is comparable to the effect on the fluorescence yield, which is decreased by a factor of 3.7 (Table I). The small discrepancy could be explained by energy storage due to the formation of long-lived triplet excited states: a quantum yield of about of 0.05 would be expected [11]. However, it is clear that at least the majority of the additional heat release due to the quenching mechanism is complete within the experimental time-window of 360 ns, and therefore that the major mechanism for the conversion of excitation energy to heat involves no long-lived excited or charge-separated state. In particular, the involvement of the triplet excited states of carotenoids can be excluded, since these generally have lifetimes from 2–10 μs [14]. The quenching mechanism also appears to be a single-photon process, since it can be observed at low pulse energies and there is no dependence of H_n on pulse energy over this range (Fig. 3). The remaining possibilities include the decay by internal conversion of the singlet excited states of chlorophyll aggregates [5] or the transfer of singlet excited states from chlorophylls to carotenoids [15] and subsequent decay by internal conversion. The specific involvement of the carotenoid zeaxanthin [16] can be excluded, however, since the conversion of violaxanthin to zeaxanthin has no effect on the fluorescence lifetime of LHC II [6].

The present result confirms that aggregation of LHC II induces a major change in its energy transfer properties which suggests a conformational change at the level of the LHC II monomer or trimer. Electron diffraction studies have provided a structure for the aggregated form of LHC II at 6 Å resolution [1]; however, the apparent functional and conformational flexibility of LHC II must be considered when relating this structure to the light-harvesting properties of LHC II in vivo.

It has been suggested that the quenching mechanism observed in vitro in aggregated LHC II may be the basis for the in vivo photoprotective mechanism known as ΔpH-dependent quenching [7,17]. This remains a possibility, since it has recently been shown using LIOAS that ΔpH-dependent quenching also results in prompt heat release (Mullineaux, C.W., Ruban, A.V. and Horton, P., unpublished data).

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