# The room temperature emission band shape of the lowest energy chlorophyll spectral form of LHCI

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Received 20 March 2003; accepted 1 June 2003

First published online 19 June 2003

Edited by Richard Cogdell

**Abstract Selective excitation, at room temperature, in the long wavelength absorption tail of the photosystem I antenna complexes, known as light harvesting complex I, induces pronounced pre-equilibration fluorescence from the directly excited pigment state. This has allowed determination of the fluorescence band shape of this low energy photosystem I chlorophyll antenna state, at room temperature, for the first time. The emission maximum is near 735 nm. The remarkable band width (55 nm) and asymmetry have never been previously reported for chlorophyll *a* states.**



*Key words:* Chlorophyll band shape; Red chlorophyll spectral state; Light harvesting complex I; Electron/phonon coupling

## 1. Introduction

An intriguing characteristic of the antenna system of photosystem I of both plants and cyanobacteria is the presence of a small number of low energy chlorophyll spectral forms or states which absorb and fluoresce at energies which are considerably lower than those of the primary reaction centre trap, P700. In plant photosystem I the steady-state excited state population is predominantly localised on these red forms at physiological temperatures [1], even though they account for only about 5–8% of the total oscillator strength. Excitation energy is transferred from them to the non-red-shifted, bulk chlorophylls, by slow, thermally activated transfer steps, which are kinetically limiting for the overall rate of photochemical trapping [2,3]. In higher plants the main function of these red forms seems to be that of light harvesting in leaves in which the light environment is enriched in wavelengths above 690 nm due to shading by other leaves (shade light) [4]. A similar function has also been suggested in dense algal mats [5].

In recent years the spectroscopic properties of these low energy chlorophyll states have excited considerable interest, with attention being directed towards the lowest lying electronic transition ($Q_y$), as this is the one involved in excitation energy transfer. The general picture which emerges is that, at least in some cases, the large bathochromic shifts come about as the result of strong excitonic interactions between chlorophyll pairs [6–8]. The coupling of pigment electrons to protein phonons is generally much stronger for the low energy states than for non-red-shifted antenna pigments [7,9–11], probably due to the greater polarisability of excited state electrons in pigment dimers with respect to monomers. Band shape calculations, in which linear electron/phonon coupling is assumed, suggest that the stronger coupling will give rise to broader absorption and fluorescence bands at physiologically relevant temperatures [12–14], though experimental data for these temperatures are absent. This point is important for the detailed modelling of energy transfer from the red forms as in the Förster mechanism the transfer rate is linear with the fluorescence (donor)/absorption (acceptor) overlap integral. In an attempt to gain information on their room temperature band shape we have analysed the steady-state emission spectra of the light harvesting complex I (LHCI) preparation of external antenna complexes of plant photosystem I [15]. LHCI was chosen for this study as much of the long wavelength emission seems to be associated with a single spectral form, which at 4 K emits maximally at 733 nm [11].

## 2. Materials and methods

LHCI was prepared from maize photosystem I and fluorescence measurements were performed using an OMA III (EG&G Instruments) as previously described [15]. Excitation wavelengths were selected with a monochromator (Heath Instruments) with bandwidths in the 1–2 nm range depending on the wavelength. When excitation was into the same band as that being detected in emission measurements it was necessary to correct for the excitation scattering spike. A correction for this was achieved by subtraction of the scattering spike measured for the same sample in the presence of a saturating concentration (80 μM) of the fluorescence quencher dibromothymoquinone. This correction was effective in eliminating most of the scattering spike.

## 3. Results and discussion

In Fig. 1 two spectra, E(λ) and PE(λ), are presented. The E spectrum was obtained with non-selective excitation at 470 nm and very similar spectra have been measured for most other excitation wavelengths used (e.g. 440 nm, 520 nm, 680 nm). As these excitation wavelengths cover absorption by all the pigment types present in the LHCI preparation (chlorophyll *a*, chlorophyll *b*, carotenoids) we consider that this is a good representation of the emission spectrum arising from a thermally equilibrated excited state distribution. As previously described [15], the E spectrum maximum at 720 nm is due to the red-shifted spectral forms, while the 685–690 nm struc-

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0014-5793/03/$22.00 
doi:10.1016/S0014-5793(03)00687-2

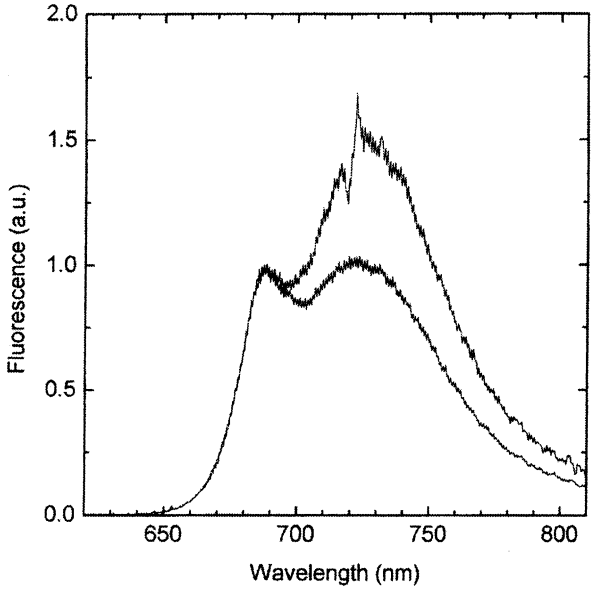


Fig. 1. Fluorescence emission spectra of LHCI. Lower curve: E spectrum, measured with the excitation wavelength at 470 nm. Upper curve: PE spectrum, measured with the excitation wavelength at 720 nm and normalised to the E spectrum in the 685–690 nm interval. The discontinuity in the PE spectrum at 720 nm is due to the residual excitation spike after correction by use of the fluorescence quencher method (see Section 2).

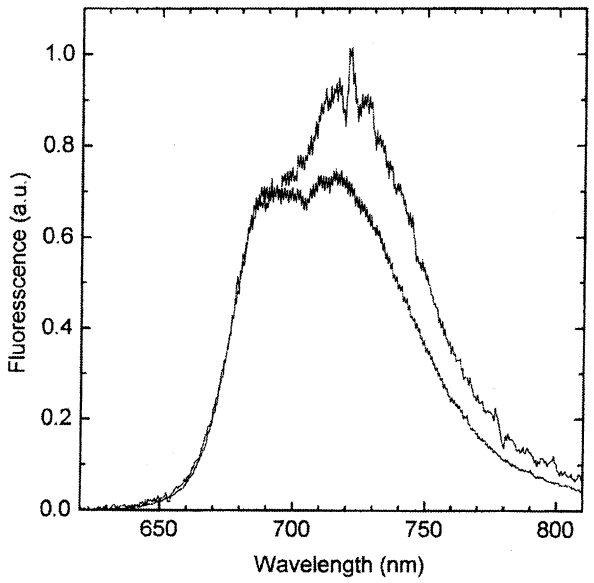


Fig. 2. Fluorescence emission spectra of the in vitro reconstituted Lhca4. Lower curve: Spectrum measured with the excitation wavelength at 500 nm. Upper curve: Spectrum measured with the excitation wavelength at 720 nm. Spectra are normalised in the 685–690 nm interval. For further details see legend to Fig. 1.

ture is associated with the non-red-shifted, bulk pigments. On the other hand, the PE spectrum was obtained by selectively exciting into the low energy absorption state(s) of LHCI at 720 nm and normalising to the E spectrum at the bulk emission structure (685–690 nm). The very marked long wavelength fluorescence with respect to the E spectrum is evident. In principle this could be due to sample heterogeneity, in which only some complexes bind long wavelength forms, as has in fact been suggested [11]. We can, however, exclude this possibility as a very similar result is obtained (Fig. 2) upon long wavelength excitation of the preparation of in vitro reconstituted Lhca4 monomer, described previously by Croce et al. [16]. On the other hand, as the long wavelength excitation is about 4 $k_BT$ below the bulk chlorophyll absorption, it is not unreasonable to suggest that this long wavelength emission is pre-equilibrium fluorescence due to slow, energetically uphill energy transfer to the bulk pigments. This interpretation implies that spectral equilibration of the red forms is slow or at least contains slow components. This has in fact been shown to be the case, on the 100–200 ps time scale, for PSI-200 [2]. We have confirmed that this is also the case for the LHCI preparation as well as reconstituted Lhca4 using single photon counting, time-resolved fluorescence measurements (unpublished data). Significant bulk/red form spectral evolution occurs on a time scale above 200 ps. These observations, when taken together, suggest that the difference between the E and PE spectra is due to pre-equilibration fluorescence from the directly excited low energy chlorophyll state(s) which equilibrates slowly with the bulk pigments. From the present data it is not possible to determine this transfer time though time-resolved experiments with this aim are being attempted.

While the E spectrum may be represented as a linear combination of the emission band shapes of all spectral states, $E_n(\lambda)$, weighted by their respective equilibrium excited state population terms, $P_n$, i.e. $E(\lambda) = \sum_n P_n E_n(\lambda)$, the pre-equilibrium spectrum (PE(λ)) contains an additional term due to emission from the low energy state(s) which are directly excited, i.e. $PE(\lambda) = \sum_n P_n E_n(\lambda) + \sum_n P^*_n E_n(\lambda)$, where $P^*_n$ is a phenomenological term which represents the pre-equilibrium population of the emission states, directly excited by the excitation beam, integrated over their excited state lifetime. The simple difference spectrum (PE(λ) minus E(λ)) thus yields the emission band shape(s) of the long wavelength state(s) which are directly excited, weighted by their pre-equilibrium excited state population. In the special case that only a single spectral form absorbs at the wavelength of the excitation beam then

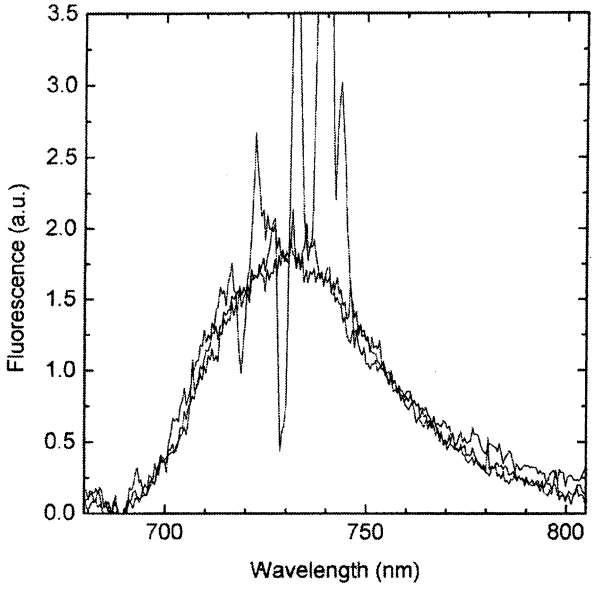


Fig. 3. Difference fluorescence spectra PE(λ) minus E(λ). For PE(λ) excitation was at 720, 730 and 740 nm. The E(λ) excitation was at 470 nm. The difference spectra are normalised at 760 nm.

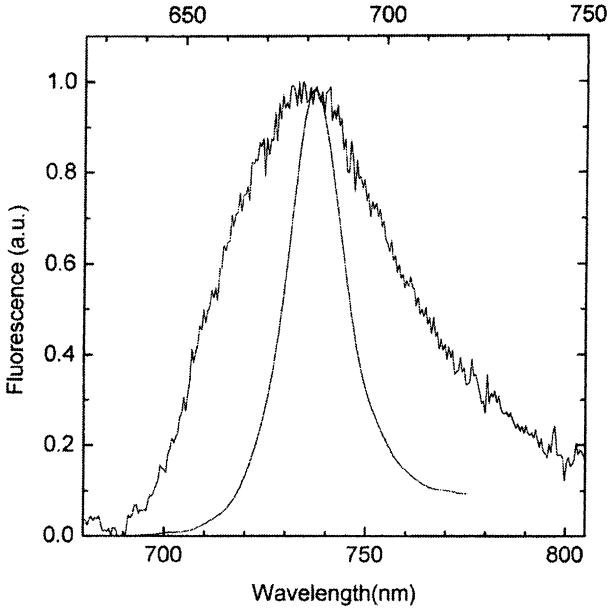


Fig. 4. The emission band shape, at 281 K, of the long wavelength fluorescence form of LHCI (F735 nm) as obtained by summation of the three spectra which are overlaid in Fig. 2. The summation procedure is described in the text. For comparison the emission spectrum of CP29, measured at 285 K, is also shown (smooth curve). The lower wavelength scale refers to the F735 nm spectrum and the upper wavelength scale to CP29.

only the relative $P^*_n$ term has a non-zero value and the PE(λ) minus E(λ) difference spectrum yields the emission band shape of this state. That this is in fact the case for these measurements with LHCI is demonstrated in Fig. 3, where the PE minus E spectra are overlaid, after normalisation to the number of counts at 760 nm, for excitation at 720 nm, 730 nm and 740 nm. It is evident that, within the measurement errors, these spectra have nearly identical band shapes.

In order to define this spectral band shape with greater accuracy we averaged these spectra in the following way. In the wavelength interval 690–715 nm and above 740 nm all three spectra were included in the average. In the wavelength interval between 715 and 745 nm the averaging was performed over those two spectra which did not contain the excitation spike. In this way the excitation spikes were eliminated and the resulting spectrum (Fig. 4) is expected to be a good approximation of the 281 K band shape of the lowest energy emitter in LHCI. The maximum at 281 K is at 735 ± 2 nm, in agreement with the 4 K data of Ihalainen et al. [11] and our own measurements at this temperature (unpublished observation). This emission spectrum, for a single chlorophyll *a* spectral state, has unique band shape characteristics. The broadness (FWHM = 55 nm; 1040 $cm^{-1}$) and marked asymmetry at room temperature have not previously been reported for chlorophylls as far as we are aware. In order to underline the remarkable characteristics of this red-shifted chlorophyll band shape we also present in Fig. 4 the emission spectrum for non-red-shifted chlorophyll *a* bound to the photosystem II antenna complex, CP29. This spectrum, which has a FWHM of 18 nm, is heterogeneously broadened due to contributions from several, energetically different, chlorophyll *a* spectral forms [17,18]. The emission bands of the single forms are expected to be considerably narrower [13], and probably of the order of 10–12 nm at room temperature. Thus the 735 nm emitting form of LHCI is about five times broader than for other protein-bound chlorophylls at room temperature, in general agreement with predictions made on the basis of the strong electron/phonon coupling demonstrated for red forms from cyanobacteria [7,9] using line narrowing spectroscopies at 2–4 K. As there is no appreciable change in band shape due to excitation in the spectral interval 720–740 nm, this extraordinary band width may be attributed almost entirely to homogeneous broadening (thermal broadening) of the electronic transition, with an insignificantly small contribution from the site inhomogeneous component. This point is worth underlining as it is the inhomogeneous component which dominates the band width at 4 K [11].

The marked asymmetry is also unusual for the $Q_y$ transition for chlorophylls at room temperature. In this case the ratio of the half band width of the low energy side with respect to that of the high energy side is 1.21 (Fig. 3) whereas for chlorophyll *a* in a variety of organic solvents this ratio is close to 1 (e.g. [21]). Eq. 1 describes the spectral coefficient of skew, in quantum mechanical terms, related to the linear electron/phonon coupling strength (*S*) [13,19,20]:

$$\gamma = \frac{1}{\left(S\left(\coth\frac{h\nu_m}{2\,k_B T}\right)^3\right)^{1/2}} \quad (1)$$

$\nu_m$ is the mean phonon frequency and the other symbols have their usual meaning. For both weak ($S < 1$) and strong coupling ($S = 2$–$3$) to low frequency protein phonons (e.g. 20 $cm^{-1}$) the spectral band shapes at room temperature should be substantially symmetrical with the coefficient of skew, $\gamma$, less than 0.02 [13,21] as is the case for chlorophyll dissolved in a range of organic solvents at 300 K [21]. In the present case of the 735 nm emission form, non-linear coupling (e.g. quadratic coupling) can be excluded as the band position (735 nm) is essentially unchanged between 4 and 281 K ([11], unpublished results). On the other hand, when coupling is to a single high frequency phonon mode (e.g. 90 $cm^{-1}$) the asymmetry at room temperature does in fact increase for both weak ($S = 1 \rightarrow \gamma = 0.1$) and strong coupling ($S = 2$–$3 \rightarrow \gamma < 0.06$), though the skew coefficient values still remain much lower than that determined for the 735 nm emitting form ($\gamma = 0.4$). Thus, for the 735 nm emission band, coupling to a single frequency mode is unable to explain the asymmetry. We therefore suggest that the explanation of the marked asymmetry may well lie in the coupling of pigment electrons to both low and high frequency phonons, which can give rise to asymmetrical bands even at room temperature [13] and of course would contribute to this remarkable band broadening. This suggestion is not novel as Ratsep et al. [7] have presented hole burning evidence at 2 K for electron coupling to both high and low frequency phonon modes for a red form in the cyanobacterium *Synechocystis* sp. Unfortunately the low energy chlorophyll of LHCI is not sensitive to hole burning [11] and so this possibility cannot be ascertained directly.

The present data clearly demonstrate that at room temperature the longest wavelength chlorophyll form of the isolated external antenna of PSI, LHCI, has its peak emission value near 735 nm. This is not, however, the longest wavelength form of the intact photosystem as this was concluded to be near 740 nm by both steady-state and time-resolved fluores-

cence measurements [1,2]. As there is no evidence for such a low energy state in the core complex [15] we suggest that it may well arise from interactions between the core and its external antenna complex(es).

In concluding we wish to point out that in the case that the PE minus E difference is due to sample heterogeneity and not to pre-equilibrium fluorescence, as we suggest, our conclusions concerning the F735 band shape would be unchanged. This is because, as can be seen from Fig. 1, the bulk emission spectra of the different subpopulations are essentially identical and Ihalainen et al. [11] have demonstrated that only one long wavelength form (F735) is present in this LHCI preparation.

*Acknowledgements:* The Lhca4 sample was kindly provided by Prof. R. Bassi and Dr T. Morosinotto of the University of Verona, Verona, Italy. This work was partially financed by the grant FIRB RBAU01E3CX.

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