DETECTION OF ALLOPHYCOCYANIN IN PHOTOSYSTEM I PREPARATIONS FROM THE BLUE–GREEN ALGA, *CHLOROGLOEA FRITSCHII*

Carole A. PULLIN, Robert G. BROWN and E. Hilary EVANS
Biology and Chemistry Divisions, Preston Polytechnic, Corporation Street, Preston, PR1 2TQ, Lancs., England

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1. Introduction

Photosystem I particles have been prepared from higher plant and eukaryotic algal chloroplasts and prokaryotic blue–green algae by essentially similar techniques [1,2]. Photosynthetic membrane of blue–green algae have been extracted with sodium dodecyl sulphate [3,4] Triton X-100 [5,6] or digitonin [7] and the resulting extracts purified via ammonium sulphate precipitation and hydroxylapatite chromatography [3,4] or by centrifugation and DEAE-cellulose chromatography [5–7]. All these extracts have shown photosystem 1 activity with ratios of P700 : chlorophyll of 1 : 40–45, although some differences in protein composition have been noticed. The composition of blue–green algal photosystem I particles has been considered [1–7] essentially similar to particles derived from higher plant eukaryotic chloroplasts, with respect to pigment composition, only chlorophyll *a* and carotenoids, mainly β-carotene [1,4,7] being present. Blue–green algae differ in gross pigment composition from higher plants and eukaryotic algae due to the presence of phycocyanins and the absence of chlorophyll *b* (see [8]). C-phycocyanin is the major phycocyanin component of *C. fritschii*, with trace amounts of allophycocyanin present [8]. It has been suggested that allophycocyanin is a bridging pigment in energy transfer between phycobilisomes and chlorophyll [9].

The presence of allophycocyanin in photosystem I particles prepared from the blue–green alga *Chlorogloea fritschii* is reported here, thus suggesting that there may be a difference between the composition of reaction centres from this blue–green alga and other higher plant and algal preparations.

2. Materials and methods

Chlorogloea fritschii (culture collection of Algae and Protozoa no. 1411/1; Dept Botany, Downing St, Cambridge) was grown and maintained as in [10].

Photosystem I particles were prepared from *C. fritschii* by a procedure similar to that in [11], involving treatment with both digitonin and Triton X-100, differential centrifugation and sucrose gradient purification. The preparation only differed from this procedure in two respects. Initially cells were washed with buffer (0.06 M Tris–HCl, 0.05 M mannitol, 0.03 M ethylenediamine tetracetic acid (EDTA), (pH 7.8)) and passed through a French press at 10 tons pressure prior to treatment with digitonin. The final purification step involved passage of the preparation down a Sepharose 6B column, eluted with 0.06 M Tris–HCl containing 0.2% Triton X-100. Photosystem I particles were assayed by P700 activity and had a ratio of P700 : chlorophyll *a* of 1 : 35–40.

All absorption changes were measured using a Pye-Unicam SP8-100 spectrophotometer. P700 were estimated as in [12]; chlorophyll *a* was extracted and estimated as in [13].

Fluorescence spectra were measured at room temperature using a modular fluorimeter manufactured by Applied Photophysics Ltd, London. Actinic light supplied by a 50 W tungsten–halogen lamp was passed through a monochromator, and sample fluorescence

measured at right angles to the excitation beam (after passage through a second monochromator) by an EMI 9816 KB photomultiplier and subsequent amplification.

3. Results and discussion

Fluorescence spectra of the photosystem I particles derived from *C. fritschii* are shown in fig.1. The level of emission is low, as reported for other preparations

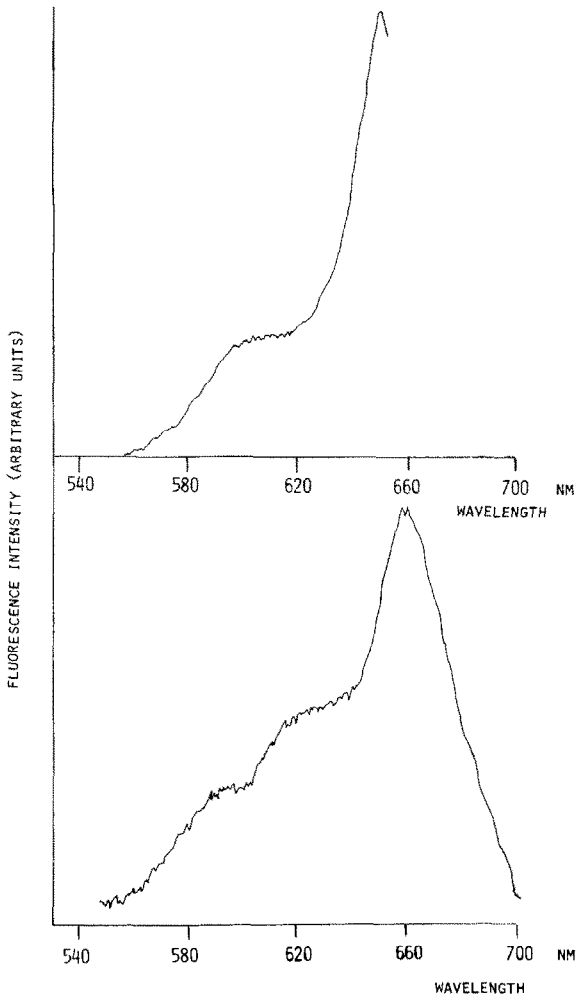

Fig.1. Fluorescence emission spectra of photosystem I particles excited at 600 nm (a) and 440 nm (b). Excitation bandwidth 5 nm; emission bandwidth 2 nm; 0.05 μg/ml chl. *a*.

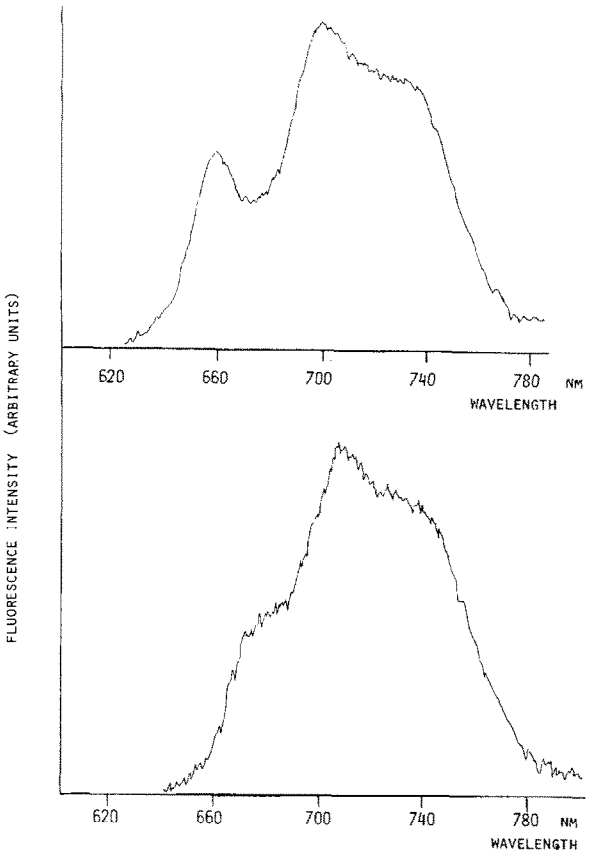

Fig.2. Excitation spectra of fluorescence of photosystem I particles at 660 nm (a) and 730 nm (b). Excitation bandwidth 2 nm; emission bandwidth 20 nm; 0.02 μg/ml chl. *a*.

[1,2] and when excited with 440 nm light showed a broad spectrum with a shoulder at 670 nm, a peak at 700 nm and a shoulder at 730 nm. If the fluorescence was excited using 600 nm light, however, an additional fluorescence band was observed at 660 nm. Excitation spectra for these fluorescence spectra are shown in fig.2; the fluorescence peaks at 660 nm and 730 nm showing considerable differences. The excitation spectrum for the 660 nm fluorescence corresponds to allophycocyanin, found only in trace amounts in *C. fritschii* [12]. The absorption spectrum of these photosystem I particles is similar to those measured in [1,4,5,7] showing no evidence for the presence of allophycocyanin. However a very small amount of allophycocyanin would be masked by the presence of the large absorption due to chlorophyll *a*. For the

same reason it is difficult to assign a contribution from allophycocyanin in the excitation spectrum of the 730 nm fluorescence.

Allophycocyanin was by the lipid extraction method in [15] which solubilised all pigment components. A mixture of allophycocyanin, extracted from *C. fritschii* and photosystem I particles clearly separated on the Sepharose 6B column. Fluorescence excitation and emission spectra were measured of the photosystem I particles, allophycocyanin and the mixture. Following separation on the column, these measurements were repeated on the allophycocyanin and photosystem I fractions, and the characteristics of the latter were found to be unchanged by the process, retaining only that amount of allophycocyanin fluorescence seen previously. This again suggests that the allophycocyanin detected in the photosystem I particles was firmly bound.

Thus it appears that a phycocyanin, allophycocyanin, normally present in trace amounts in blue–green algae [14] was present in these photosystem I particles, as detected by its fluorescence. Whether the allophycocyanin is involved in the photochemistry of these photosystem I preparations remains to be determined.

Acknowledgements

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