Biochimica et Biophysica Acta, 292 (1973) 858–861

BBA Report

BBA 41231

Energy transfer in phycobilisomes from phycoerythrin to allophycocyanin

E. GANTT and C.A. LIPSCHULTZ

Smithsonian Institution, Radiation Biology Laboratory, 12441 Parklawn Dr., Rockville, Md. 20852 (U.S.A.)

(Received February 19th, 1973)

SUMMARY

Allophycocyanin appears to be the pigment through which energy trapped by phycobiliproteins is funneled to the chloroplast lamellae. Isolated, intact phycobilisomes from *Porphyridium cruentum* have a maximum fluorescence emission peak at 675–680 nm when excited at 545 nm. Upon dissociation, when the energy transfer is interrupted the 675–680-nm peak declines. Excitation at 435 nm produced no significant fluorescence at this wavelength.

Phycobilisomes from *Porphyridium cruentum* consist of phycobiliproteins which serve as light harvesting pigments. From *in vivo* fluorescence studies it has been shown that the light energy absorbed by phycobiliproteins is ultimately transferred to chlorophyll a[1–3] where it is converted to chemical energy. It has been generally assumed that energy absorbed by phycoerythrin is passed through phycocyanin to chlorophyll. Recently we reported the existence of allophycocyanin in *P. cruentum* phycobilisomes[4]. We now report that allophycocyanin appears to be the key pigment in the transfer chain and that it serves as the funnel through which energy transfer occurs in phycobilisomes.

Phycobilisomes from cells of *P. cruentum* grown under continuous illumination were isolated in a 0.5 M phosphate buffer–sucrose gradient system[4], modified to eliminate the occasional, slight chlorophyll contamination. Upon analysis the phycobilisomes were found to be composed of several phycobiliproteins (Table I). The phycoerythrins accounted for at least 80% of the phycobilisome content, while the remainder consisted of R-phycocyanin in about equal proportions (details to be published separately). The maximum absorption and fluorescence peaks of B-phycoerythrin and allophycocyanin are essentially identical to those reported by French *et al.*[5]. R-Phycocyanin, however, contains both a red

TABLE I

MAJOR ABSORBTION AND EMISSION PEAKS OF PHYCOBILIPROTEINS FROM TWO RED ALGAE
Pigment determinations of *P. cruentum* were made at room temperature in 0.01 M phosphate buffer (pH 6.8) with a Cary 14 spectrophotometer and an Aminco–Bowman spectrofluorometer. The data for *P. naidum* were from French *et al.*[5].

Absorbtion	*Porphyridium cruentum*		*Porphyra naidum*	
	Absorbtion (λ nm)	*Emission (λ nm)*	*Absorbtion (λ nm)*	*Emission (λ nm)*
B-Phycoerythrin	545	575	546	576
b-Phycoerythrin	545	570	–	–
R-Phycocyanin	555 or 617	636	–	–
Phycocyanin	–	–	616	637
Allophycocyanin	650	660	654	663

and a blue chromophore and its fluorescence emission peak occurs at about 636 nm regardless of the excitation wavelength (555 or 617 nm). A phycocyanin with a single absorption maximum at 616 nm such as is present in *Porphyra* has not been found in *P. cruentum.*

The major fluorescence emission peak occurred at about 675–680 nm (Fig.1A) when intact phycobilisomes (0.5 M phosphate buffer, pH 6.8) were excited at 545 nm. The emission at 575 nm was considerably lower and is assumed to be largely due to free phycoerythrin in the preparation. When phycobilisomes were suspended in 30 mM phosphate buffer (pH 6.8) there was an immediate and continuing increase in phycoerythrin fluorescence (575 nm) (Fig.1B) and a concomitant decrease of the 675–680-nm fluorescence peak. After 3 h there were no additional detectable changes in the fluorescence emission peaks under these conditions, presumably because the phycobiliproteins of the phycobilisomes were uncoupled and no longer able to transfer energy. The decrease of the fluorescence peak of allophycocyanin cannot be explained by a simple degradation of the pigment because there was not an equivalent decrease in the absorption peak of allophycocyanin (650 nm) during the 3-h period.

The 675–680-nm emission peak in the intact phycobilisomes is assumed to be allophycocyanin, because of the four phycobiliproteins comprising the phycobilisomes, its fluorescence emission is at the longest wavelength. It is not likely, though possible, that chlorophyll is responsible for the 675–680-nm fluorescence for two reasons: chlorophyll was virtually undetectable (by absorption, of fluorescence with 80% acetone extraction), and when phycobilisome preparations were excited at 435 nm the resulting fluorescence was very low (less than 4%) (Fig.1A). Bergeron[6] showed that in *Anacystis nidulans* (which appears to lack allophycocyanin) phycocyanin in the monomer form fluoresced at 660 nm, whereas the aggregated form fluoresced at 680 nm. It seems plausible that allophycocyanin, analogous to phycocyanin, fluoresces at 675–680 nm in the aggregated state, even though a purified pigment solution (monomer) fluoresces at 660 nm.

The demonstrated high efficiency of energy transfer (greater than 80%) by the

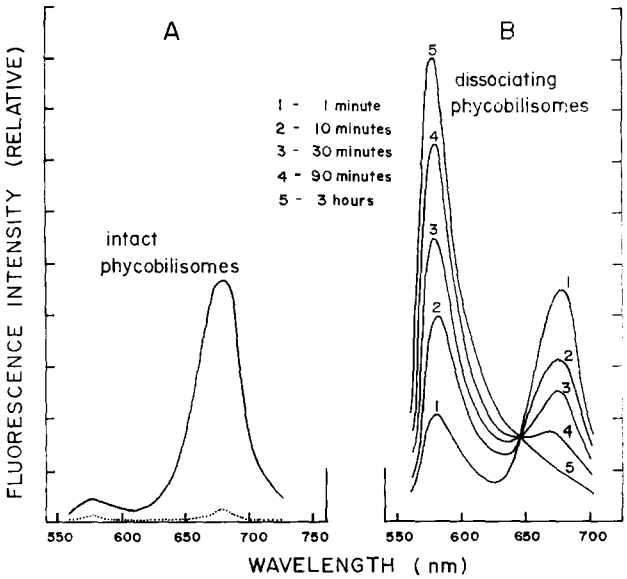

Fig.1. Fluorescence emission spectra of intact and dissociating phycobilisomes from *P. cruentum* ($A_{545\,nm} \approx 0.12$) with exciting wavelength at 545 nm (——) and 435 nm (. . . . .). (A) Intact phycobilisomes in 0.5 M phosphate buffer (pH 6.8). The major emission occurs at 675–680 nm (assumed to be due to allophycocyanin), and a secondary emission at phycoerythrin (575 nm). Excitation at 435 nm causes a very small amount of fluorescence (less than 4%), easily attributable to phycobiliprotein absorption at that wavelength. (B) Phycobilisome preparation in 30 mM phosphate buffer (pH 6.8). Dissociation began immediately and was followed from 1 min to 180 min. Spectra were made at room temperature with an Aminco–Bowman spectrofluorometer equipped with an off-axis ellipsoidal mirror condensing system, and an R136 photomultiplier tube (Hamamatsu TV). The band pass on the excitation side was 11 nm, and 2.7 nm on the emission side. Spectra were corrected after determining the lamp output and the emission grating-phototube efficiency.

phycobiliproteins *in vivo*[1–3] is possible primarily because these pigments exist in phycobilisomes which are highly organized and compact structures directly attached to the photosynthetic lamellae[7–9]. When the spatial organization within the phycobilisomes is destroyed the energy transfer is interrupted. A structural phycobilisome model, which has emerged from data in our laboratory, consists of an allophycocyanin core in direct contact with the photosynthetic lamellae, and an extensive phycoerythrin shell surrounding the core. Thus, energy absorbed primarily by the phycoerythrins and R-phycocyanin would be funneled *via* allophycocyanin (emission 675–680 nm in the aggregated form) to chlorophyll *a* and to the reaction center. Therefore, allophycocyanin appears to be a much more important pigment in photosynthesis than has heretofore been assumed.

This work was supported in part by the U.S. Atomic Energy Commission Contract No. AT-(40-1)-4310 and the Smithsonian Institution. Published with the approval of the Secretary of the Smithsonian Institution.

REFERENCES

1 Duysens, L.N.M., (1952) *Transfer of Excitation Energy in Photosynthesis,* Ph.D. Thesis, Utrecht
2 French, C.S. and Young, C.K. (1952) *J. Gen. Physiol.* 35, 873–890
3 Tomita, G. and Rabinowitch, E. (1962) *Biophys. J.* 2, 483–499
4 Gantt, E. and Lipschultz, C.A. (1972) *J. Cell Biol.* 54, 313–324
5 French, C.S., Smith, J.H.C., Virgin, H.I. and Airth, R.L. (1956) *Plant Physiol.* 31, 369–374
6 Bergeron, J.A. (1963) in *Photosynthetic Mechanisms of Green Plants,* pp. 527–536, Natl. Acad. Sci., Washington, D.C.
7 Gantt, E. and Conti, S.F. (1966) *Brookhaven Symp. Biol.* 19, pp. 393–405
8 Gantt, E. and Conti, S.F. (1966) *J. Cell Biol.* 29, 423–434
9 Clement-Metral, J.D. and Lefort-Tran, M. (1971) *FEBS Lett.* 12, 225–228