Vol. 81, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 30, 1978 Pages 571-575

EXCITATION ENERGY TRANSFER IN *ANACYSTIS NIDULANS*

K. Csatorday, J.W. Kleinen Hammans[+] and J.C. Goedheer[+]

Institute of Plant Physiology, Biological Research Center, Hungarian Academy of Sciences, 6701 Szeged, Hungary.

[+]Biophysics Research Group, State University of Utrecht, Princetonplein 5, Postbus 80.000, 3508 TA Utrecht, The Netherlands.

Received January 6,1978

Summary: The fate of excitation energy was investigated in phycocyanin-rich and phycocyanin-deficient cells of *Anacystis nidulans*. It was shown that direct energy transfer between phycocyanin and chlorophyll *a*, bypassing the excitation energy transfer chain through allophycocyanin and allophycocyanin B in the phycobilisome, may exist. The presence of allophycocyanin B in the fluorescence spectrum was demonstrated.

Introduction: Phycobiliproteins are special pigments present in the phycobilisomes of red and blue-green algae; their function is to harvest light energy and transfer it to the reaction center of PS II via chlorophyll *a* in the photosynthetic membrane (1-4). When the reaction centres are closed and thus unable to accept additional energy the excess energy is given off as fluorescence of the light-harvesting pigment complex. This process involves uphill energy transfer between the reaction center and the light harvesting pigments (5, 6, 7). The shoulder at 660 nm in the delayed fluorescence spectrum is attributed to allophycocyanin. The peak in the DCMU-induced difference spectrum of fluorescence is located at 683 nm and indicates the presence of allophycocyanin B which causes the blue shift of the 685 chlorophyll *a* peak. Recent investigations on isolated phycobilisomes of *Porphyridium cruentum* show (8), that allophycocyanin B (9) is present and could be involved in energy transfer between the phycobilisomes and chlorophyll *a*.

Materials and Methods: *Anacystis nidulans* was grown at 39°C in medium C described in (10). The cultures received high intensity illumination (5000 $\mu W.cm^{-2}$) from fluorescent tubes and were flushed by air enriched with 5% CO_2.
Fluorescence was measured with a laboratory-built apparatus employing

0006-291X/78/0812-0571$01.00/0

a Bausch and Lomb 500 nm monochromator (slit width 1 mm) and a liquid nitrogen cooled Philips XP 1005 photomultiplier. Excitation was provided by a 150 W slide projector combined with neutral filters and either a Schott 1 mm VG 9 (green) or BG 7 (blue) filter.
A 3 cm cuvette with 6% $CuSO_4$ solution was also used in the light path. The excitation light was chopped and the fluorescence was detected using a lock-in amplifier. The spectral characteristics of the monochromator-photomultiplier combination in the region of interest, 650-700 nm, were such, that on no 10 nm segment did the change in the correction factor exceed 1% and thus the spectra were not corrected for the spectral sensitivity and transmission efficiency of the apparatus. In the experiments "high intensity" illumination corresponds to 5700 $\mu W.cm^{-2}$; "low intensity" to 360 $\mu W.cm^{-2}$.
O_2 production was measured as described earlier (11).
All measurements were carried out at room temperature.

Results and discussion: Fig. 1 shows the absorption spectrum of a vigorously growing young culture of *Anacystis nidulans* as well as that of a yellow-green culture obtained by ageing. The spectra show that the yellow-green culture has lost most of its phycocyanin as compared to its chlorophyll content.
Action spectra of O_2 evolution for the two samples are shown in Fig. 2. The curves are presented in such scale that the contribution of chlorophyll absorption in one matches that in the other.
A qualitative assessment indicates that when most of the phycocyanin is lost, its contribution in the action spectrum of O_2 evolution has decreased by only a factor of three. This means that the greater part of the phycocyanin molecules is not essential as far as photosynthetically important energy transfer is concerned. The shoulder at 650 nm in both spectra corresponds to allophycocyanin absorption (12, 13) and seems to be unaffected by the change in the phycocyanin content.

Fluorescence spectra of the two types of cultures are presented in Fig. 3. Excitation in the green -PS II light- and blue -PS I light- spectral regions respectively, was employed for both samples. The fluorescence spectrum of the young phycocyanin-rich culture excited by green light has maxima at 684,5 nm and at 656 nm corresponding to the fluorescence of chlorophyll *a* and phycocyanin, respectively. The presence of allophycocyanin fluorescence may be the cause for the red shift of the phycocyanin peak. Blue excitation results in a distinctive maximum at 685 nm, a slight shoulder at 680 nm and a prominent one at 655 nm.

The fluorescence spectra of the yellow-green culture show that the fluorescence attributable to phycocyanin is indeed absent, the maximum is at 680 nm with shoulders discernible at 685 nm and

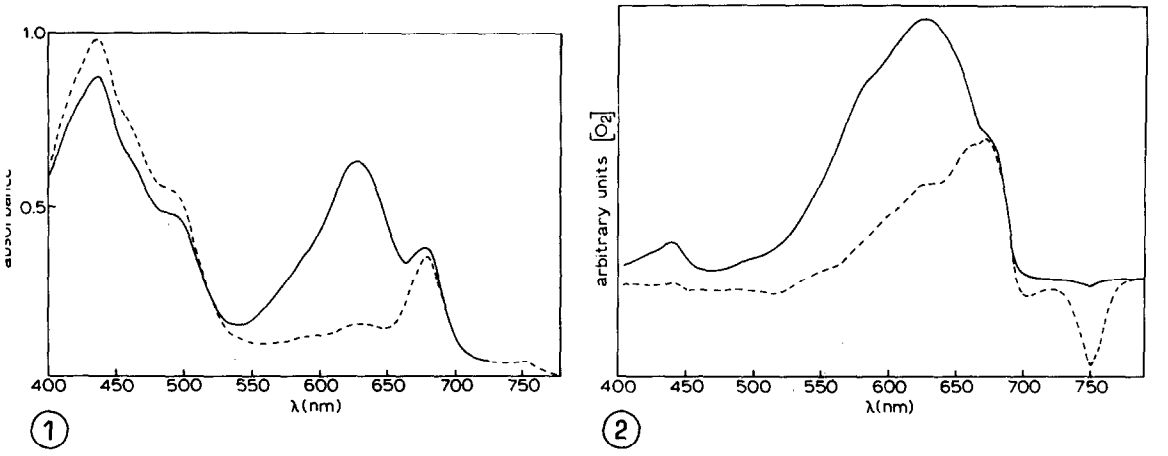

Fig. 1 Absorption spectra of phycocyanin-rich (——) and phycocyanin-deficient (----) cultures of Anacystis nidulans.

Fig. 2 Action spectra of O_2 production of the phycocyanin-rich (——) and the phycocyanin-deficient (----) culture of Anacystis nidulans.

at 662-663 nm. Excitation by blue light, absorbed mainly by chlorophyll *a*, gives a fluorescence peak at 684,5 nm and a shoulder at 680 nm. The 680 nm component in the fluorescence spectra of whole cells is attributable to allophycocyanin B. This pigment has been isolated from *Anacystis* (9), but it is present in very small amounts and can be seen in the fluorescence spectrum only as a slight shoulder or deduced from the slight blue-shift of the chlorophyll *a* peak.

The concomitant drop in phycocyanin and chlorophyll fluorescence upon green excitation of the phycocyanin-deficient yellow-green culture with the subsequent manifestation of allophycocyanin and allophycocyanin B fluorescence implies that in normal, phycocyanin-rich, cultures there is considerable and direct excitation energy transfer between phycocyanin and chlorophyll *a*, bypassing the route via allophycocyanin and allophycocyanin B. Were it not the case it would be expected that chlorophyll *a* fluorescence would still predominate over allophycocyanin B fluorescence, all the more so since the efficiency of energy transfer between the two is presumed to be very high (8).

The ratios of fluorescence excited by high intensity light F_{max} to that excited by low intensity light F_{min} with most of the

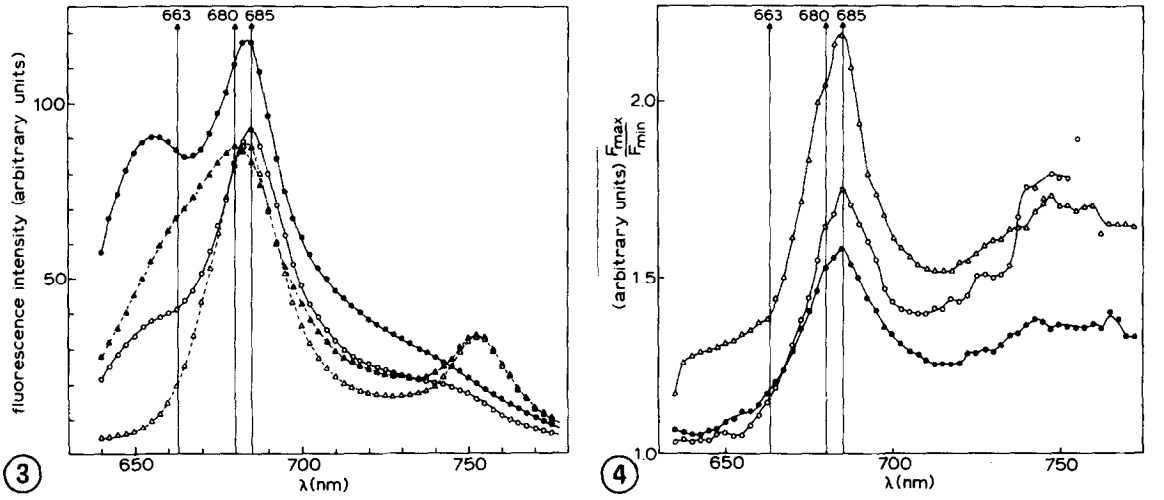

Fig. 3 Fluorescence spectra of the phycocyanin-rich (circles) and phycocyanin-deficient (triangles) cultures excited by green light (● ▲) and blue light (○ △) of saturating intensities 5700 $\mu W.cm^{-2}$ and 2750 $\mu W.cm^{-2}$ respectively.

Fig. 4 Spectral distribution of ratios $\frac{F_{max}}{F_{min}}$ of fluorescence excited by high intensity green light (5700 $\mu W.cm^{-2}$) to that excited by low intensity green light (360 $\mu W.cm^{-2}$)

reaction centers of PS II in the closed and open state respectively are shown in Fig. 4 for three cultures of different age. The maximum is located at 685 nm and a prominent shoulder at 680 nm indicates considerable transfer of excitation energy to allophycocyanin B. The shoulders at 662-663 and 650 nm are attributed to allophycocyanin and phycocyanin respectively, and have been observed in delayed fluorescence spectra as well (5). The presence of the 680 nm component has been inferred from the blue shift of the 685 nm chlorophyll *a* fluorescence peak (5) but in our experiments it is manifested quite distinctly as a shoulder or a peak with the chlorophyll peak remaining at 685 nm.

Recent studies on isolated phycobilisomes (8) have demonstrated that excitation energy is conveyed to allophycocyanin B, and the suggestion has been made that through this pigment the energy may be funnelled to chlorophyll *a*.

Our data suggest that in intact cells of *Anacystis* the possibility of a direct transfer between phycocyanin and chlorophyll should be considered as well.

REFERENCES

1. Gantt, E., and Conti, S.F. (1966) J. Cell Biol., 29, 423-434.

2. Gantt, E., and Conti, S.F. (1969) J. Bacteriol., 97, 1486-1493.

3. Gantt, E., and Lipschultz, C.A. (1973) Biochim. Biophys. Acta 292, 858-861.

4. Gantt, E., Lipschultz, C.A., and Zilinskas, B. (1976) Biochim. Biophys. Acta 430, 375-388.

5. Murata, N. (1977) Plant & Cell Physiol., Special Issue No.3, 9-13.

6. Wang, R.T., and Myers, J. (1977) Plant & Cell Physiol., Special Issue No.3, 3-7.

7. Ley, A.C., and Butler, W.L. (1977) Plant & Cell Physiol., Special Issue No.3, 33-46.

8. Ley, A.C., Butler, W.L., Bryant, D.A. and Glazer, A.N., (1977) Plant Phys., 59, 947-980.

9. Glazer, A.N., and Bryant, D.A. (1975) Arch. Microbiol., 104, 11-22.

10. Kratz, W.A., and Myers, J. (1955) Amer. J. Bot., 42, 282-287.

11. Kleinen Hammans, J.W., Hendriks, G.M., and Teerlink, T. (1977) Biochem. Biophys. Res. Comm., 74, 1560-1565.

12. Halldal, P. (1970) Photobiology of Microorganisms ed. Halldal, P. pp. 17-55, Wiley-Interscience, London.

13. Lemasson, C., Tandeau de Marsac, N., and Cohen-Bazire, G. (1973) Proc. Nat. Acad. Sci. USA, 70, 3130-3133.