

BBA Report

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FLUORESCENCE FROM SENSITIZING PHYCOBILIN CHROMOPHORES IN THE BLUE-GREEN ALGA *ANACYSTIS NIDULANS*

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Summary

Regeneration of the pigment system of *Anacystis nidulans* was studied following nitrate starvation. Three new, distinct fluorescence bands, at 596, 615 and 636 nm attributed to sensitizing phycobilin chromophores were detected. They each possess a separate excitation band at 425, 395 and 410 nm, respectively.

The blue-green alga *Anacystis nidulans* contains C-phycocyanin as the main accessory pigment. Studies of chromatographically pure C-phycocyanin fluorescence, fluorescence excitation and fluorescence excitation polarization spectra have led to the conclusion that at least two types of chromophores exist, a sensitizing (s) and a fluorescing (f) type [1–3]. Excitation energy transfer from the sensitizing chromophore to the fluorescing chromophore is presumed to be very efficient since no fluorescence from the sensitizing chromophore has been observed. A slight blue shift of the fluorescence spectra of purified phycobiliproteins upon changes in the protein concentration is interpreted as evidence indicating that the 's' type chromophores are capable of some fluorescence [1].

The present study was undertaken with the aim of detecting the presence of the sensitizing chromophores when excitation energy transfer to the fluorescent types is impaired due to nitrate starvation and concurrent loss of phycocyanin. Restoration of nitrate to the culturing medium results in rapid repigmentation [4]. Since excitation energy transfer efficiency is very sensitive to the integrity of the pigment system, the manifestation of fluorescence from otherwise sensitizing chromophores may be expected during the regeneration process.

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Qualitative changes in the fluorescence spectra of *A. nidulans* during regeneration of its pigment system following nitrate starvation are presented in Fig. 1. Three distinct, hitherto unreported fluorescence bands emerge at various stages of repigmentation and this indicates that at least three sensitizing chromophores, able to fluoresce at 596, 615 and 636 nm, respectively, take part in excitation energy transfer to the fluorescent (at 650 nm) phycocyanin chromophore. These disappear later as more, fluorescent, subunits are synthesized and consequently 'f' fluorescence at 650 nm increases at the expense of 's' fluorescence at shorter wavelengths. The 's' fluorescence bands each possess a distinct excitation band in the blue region of the excitation spectrum (Fig. 2). The 596, 615 and 636 nm fluorescence bands have their blue excitation bands at 425, 395 and 410 nm, respectively. The excitation band at 385 nm belongs to *P*-750 fluorescence as reported earlier [5]. The excitation spectrum for 636 nm fluorescence first peaks at 395 nm indicating energy transfer from the 615 nm chromophore (8 h) and 4 h later (12 h) peaks at 410 nm indicating a relative increase in the number of 636 nm sensitizing chromophores. To prevent any ambiguity the excitation spectrum of the 636 nm chromophore from another culture where it is better seen is also presented. For comparison the fluorescence excitation spectrum for 680 nm fluorescence of a fully re-

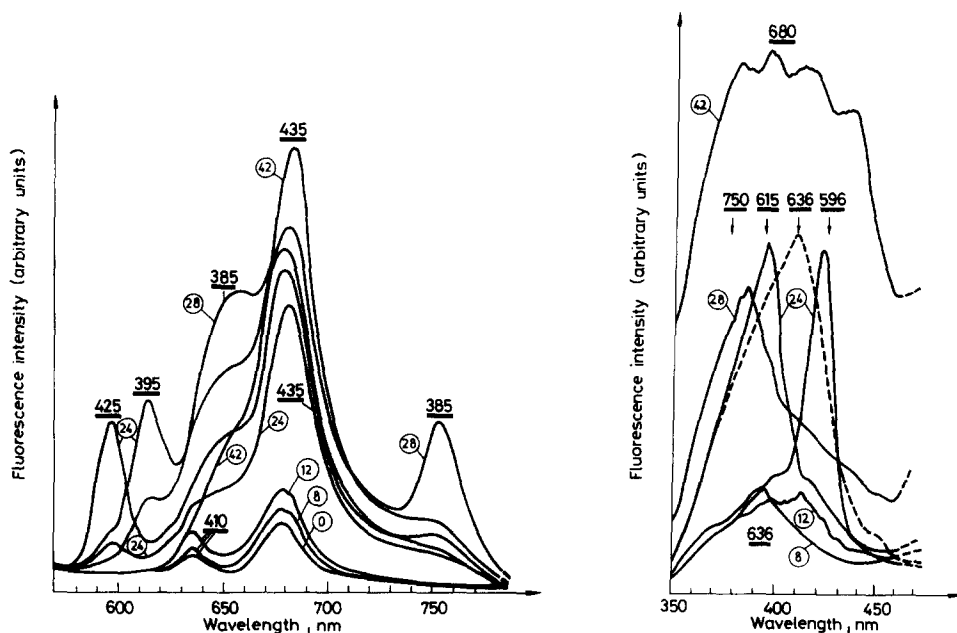


Fig. 1. Relative fluorescence spectra of *A. nidulans* samples taken during the regeneration of phycocyanin. The encircled numbers refer to the time lapsed (in hours) since restoration of nitrates, whereas underlined numbers indicate the wavelength of excitation (nm). Since the intensities of the various peaks change during the regeneration process as energy transfer becomes more efficient, the spectra were selected to show the individual fluorescence bands of the sensitizing chromophores as distinctly as possible.

Fig. 2. Fluorescence excitation spectra of *A. nidulans* samples taken during the regeneration of phycocyanin. The encircled numbers refer to the time lapsed (in hours) since restoration of nitrates, whereas underlined numbers indicate the wavelength of observation (nm). The spectrum drawn with a dashed line belongs to a different series of measurements but it is included since the 636 nm fluorescence band in Fig. 1 is of relatively low intensity.

generated sample with intact excitation energy transfer pathways is presented. The individual sensitizing chromophores are indicated by shoulders only, the one at 440 nm attributable to chlorophyll *a* absorption. The respective fluorescence spectrum (42 h) does not allow resolution into separate components owing to efficient excitation transfer between sensitizing and fluorescent chromophores.

The results presented above allow some speculation about the possible existence of other sensitizing chromophore types in phycoerythrin-containing algae as well.

References

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