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Low-temperature fluorescence emission and excitation spectra for *Anacystis nidulans*

Several investigations of the fluorescence of chloroplasts and algae cooled to 77 °K have demonstrated the existence of three distinct emission bands at approx. 690, 700, and 720 mμ (refs. 1–13). These three emissions (F690, F700, and F720) are presumed to come from chlorophyll *a* in three different environments: C_{F690}, C_{F700}, and C_{F720}. At room temperature C_{F690} appears to be responsible for the major fluorescence emission and is generally thought to be associated with System II of photosynthesis; C_{F720} is generally considered to be associated with System I. KOK[4] and GOEDHEER[7,8] have suggested that C_{F700} is associated with System I, but other workers support the idea that C_{F700} is associated with System II[2,3,5,9–13].

In blue-green (or red) algae it has been shown that the ratio (F690 + F700): F720 for excitation with red (or green) light absorbed primarily by phycocyanin (or phycoerythrin) is 2 to 4 times as high as the ratio for excitation with blue light absorbed primarily by chlorophyll *a* (refs. 3–5) (see also Fig. 1). In the present communication an assessment is made of the relative efficiencies of phycocyanin and chlorophyll *a* in sensitizing F690, F700, and F720 from the excitation spectrum for each emission. Recently MURATA, NISHIMURA AND TAKAMIYA[13] published such spectra for F690 and F720. Although our observations generally substantiate theirs, significant differences do exist between the two sets of excitation spectra.

Anacystis nidulans was cultured in the medium of KRATZ AND MEYERS[14] as described by BERGERON[3] for the studies of emission spectra. For the excitation spectra, the algae were grown at about 30° under a bank of seven F2412-CW-HO fluorescent lamps in a Brunswick incubator shaker.

Fluorescence emission spectra from a dilute suspension of *A. nidulans* are shown in Fig. 1, as the relative number of quanta emitted per unit wave number interval

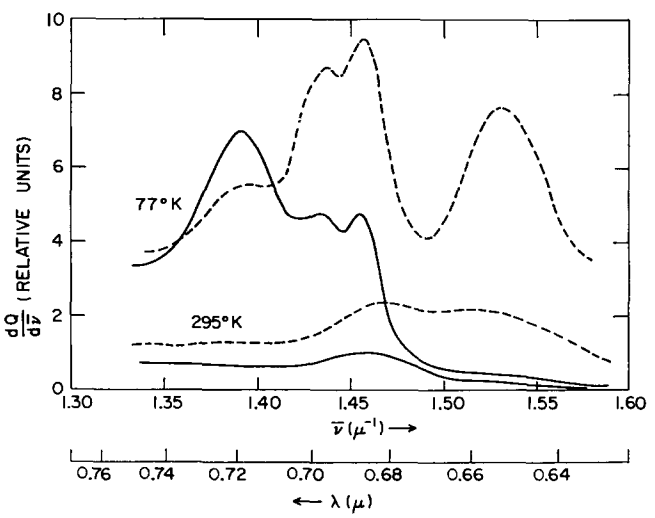

Fig. 1. Fluorescence emission spectra for a dilute suspension of 1 mm thickness at 77 °K and 296 °K. Solid curves are for 436-mμ excitation; dashed curves for 578-mμ excitation.

($dQ/d\bar{\nu}$). The fluorimeter used and the corrections applied have been described[15]. The emission bands F690, F700, and F720 appear at 687, 697 and 712 mμ respectively in Anacystis at 77 °K. (The emission band at 654 mμ belongs to phycocyanin.) For excitation with 436-mμ light absorbed predominantly by chlorophyll *a* and carotenoids, the proportion F690:F700:F720 is 1.0:1.0:2.4, based on the estimated area under each band, whereas with 578-mμ light absorbed predominantly by phycocyanin the proportion is 1.0:0.9:0.8. Thus, when chlorophyll *a* is excited directly with blue light, the relative contribution of F720 is about 3 times its relative contribution for excitation with light absorbed by phycocyanin alone.

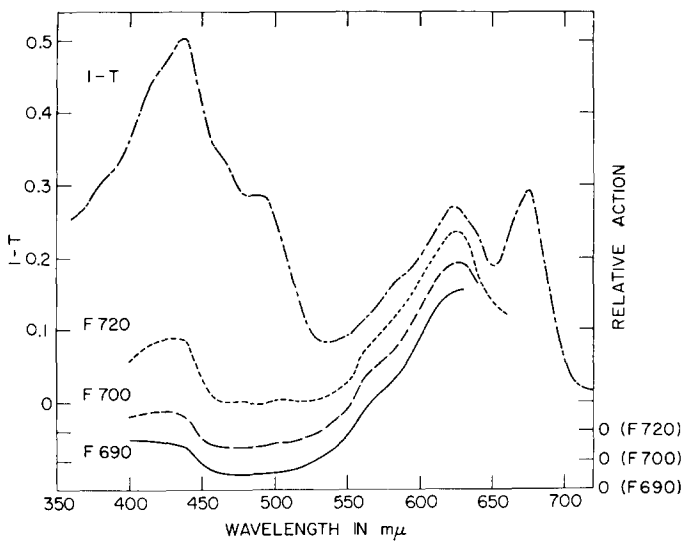

Fig. 2. Excitation spectra for a sample of 1 mm thickness at 77 °K: F690 (———), F700 (– – –), F720 (-------). Absorption spectrum (1—T) for duplicate sample at room temperature (–·—·–). All curves have been normalized at 620 mμ, and baselines have been staggered for clarity.

Fluorescence excitation spectra for F690, F700, and F720 are shown in Fig. 2 along with the absorption spectrum (1—T) of the sample at room temperature. The spectra were obtained as described elsewhere[16] and are expressed as the ratio of emission signal to quantum intensity of exciting light. Filters used to isolate F690 were a Bausch and Lomb second-order interference filter (688 mμ, $\Delta\lambda = 13$ mμ) and Corning red glass filters 2-58 and 2-64. For F700 the same red glass filters were used with another interference filter (700 mμ, $\Delta\lambda = 12$ mμ). For F720 an interference filter (725 mμ, $\Delta\lambda = 9$ mμ) was used with the Corning 2-64 and a Wratten 89B.

Comparison of the excitation spectra with the absorption spectrum shows that carotenoid absorption in the region 450 to 530 mμ contributes almost nothing to the fluorescence in question. This confirms earlier observations[13,17] of the relative ineffectiveness of carotenoids in sensitizing fluorescence in blue-green algae. Since the ineffective carotenoids compete with chlorophyll for the absorption of blue light, the relative efficiency of chlorophyll in sensitizing each fluorescent emission was estimated in the following manner. Chlorophyll *a* "action" was assumed to be proportional to the fluorescence excited by 440-mμ light (f_{440}) *minus* the fluorescence excited by

450-mμ light (f_{450}). Chlorophyll *a* absorption was assumed to be proportional to the absorption at 440 mμ ($1-T_{440}$) *minus* the absorption at 450 mμ ($1-T_{450}$). The relative efficiency of chlorophyll *a* was calculated from the ratio $(f_{440}-f_{450}):(T_{450}-T_{440})$. Similarly the relative efficiency of phycocyanin was calculated from the ratio $(f_{620}-f_{570}):(T_{570}-T_{620})$. The ratio of chlorophyll efficiency to phycocyanin efficiency in each case was estimated as

$$R = \frac{f_{440}-f_{450}}{T_{450}-T_{440}} : \frac{f_{620}-f_{570}}{T_{570}-T_{620}}$$

Values of R for F690, F700, and F720 are 0.23, 0.30 and 0.55, respectively. Values of R for F690 and F720 calculated from Fig. 7 of MURATA, NISHIMURA AND TAKAMIYA[13], are 0.10 and 0.30 respectively. However, if the ratio of chlorophyll efficiency to phycocyanin efficiency for F720 is calculated as $f_{680}(1-T_{625}):f_{625}(1-T_{680})$ from the data of MURATA, NISHIMURA AND TAKAMIYA, a value of 0.58 is obtained, which agrees well with our value of 0.55. The action spectra of MURATA, NISHIMURA AND TAKAMIYA, therefore, do not appear to be quantitative in the region of blue light absorption.

Table I shows that absorption of light by phycocyanin is more effective than absorption of light by chlorophyll *a* in sensitizing any of the low-temperature emissions (F690, F700 or F720) in *A. nidulans*. (Therefore we must disagree with MURATA, NISHIMURA AND TAKAMIYA[13] that "...chlorophyll *a* is more effective for F720 than phycobilins and chlorophyll *b*".) The relative efficiencies of chlorophyll *a* and phycocyanin for the total fluorescence emission at 77 °K suggests that a considerable fraction of the chlorophyll *a* represented in the absorption spectrum is non-fluorescent and receives little or no excitation from phycocyanin (*cf.* ref. 17) and/or that phycocyanin itself contributes more to the total emission than is generally assumed. Light absorbed by chlorophyll *a* is considerably more effective for F720 than for either F690 or F700. This result supports the idea that C_{F720} is associated with System I and that C_{F690} and C_{F700} are associated mainly with System II.

TABLE I

RELATIVE EFFICIENCIES OF PHYCOCYANIN AND CHLOROPHYLL IN SENSITIZING FLUORESCENCE

Values outside parentheses are calculated from the R values and the proportion F690:F700:F720 upon excitation with 578-mμ light. Values inside parentheses are calculated from the R values and the proportion F690:F700:F720 upon excitation with 436-mμ light. Relative efficiency of phycocyanin is arbitrarily set equal to 100.

Emission	*Phycocyanin*	*Chlorophyll a*
F690	100 (100)	23 (23)
F700	90 (76)	27 (23)
F720	80 (100)	44 (55)

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