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On the low-temperature fluorescence spectra of blue-green and red algae

With blue-green and red algae cooled to 77 °K the shape of the fluorescence spectrum is markedly dependent on the wavelength of excitation. MURATA, NISHIMURA AND TAKAMIYA[1] and BERGERON AND OLSON[2], working with *Anacystis nidulans*, found that the 685 and 695 nm bands are primarily excited by phycobilin absorption, while the 720 nm band is excited both by chlorophyll and phycobilin absorption. The 77 °K fluorescence spectrum of Anacystis, however, differs from that of various other species of blue-green and red algae in having a relatively low band around 720 nm (located in Anacystis at 715 nm). Also the ratio of the three fluorescence bands (called earlier F685, F695 and F720, *cf.* ref. 3) depends strongly on the age and growth conditions of the cells, in contrast to that of the other species.

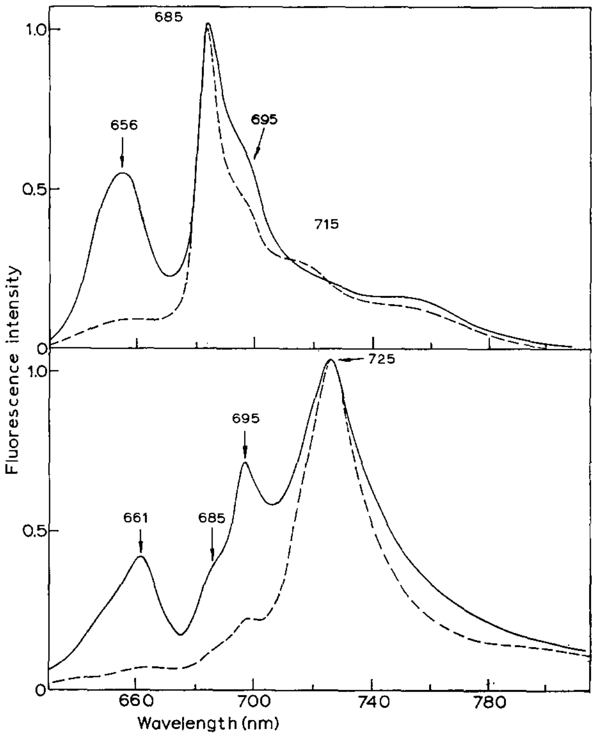

Fig. 1. Fluorescence spectrum of a 7-day-old culture of the blue-green algae *A. nidulans* (upper) and *S. cedrorum* (lower), measured at 77 °K and resulting from incident light of 547 nm (———) and 437 nm (-----).

In Fig. 1 the 77 °K fluorescence spectrum resulting from excitation at 437 nm (chlorophyll *a* absorption) and at 547 nm (mainly phycobilin absorption) is given for the blue-green algae *Anacystis nidulans* and *Synechococcus cedrorum*. For both samples, a 7-day-old culture was used, grown at 27° in a light cabinet (14 h light and 10 h dark). With Anacystis a spectrum of a 3-day-old sample showed a much more pronounced F720 band and strongly resembled that obtained by BERGERON AND OLSON[2], whereas with Synechococcus the spectrum of a 3-day-old one was identical to that shown in Fig. 1 (lower graph). With all samples photosynthetic activity could be measured with the platinum electrode. The fluorescence spectra of the blue-green algae *Oscillatoria amoena*, *Nostoc calcicola* and *Anabaena catinata*, were similar to those of Synechococcus. All showed a high band between 715 and 730 nm, when excited with 437 nm light. Except with some samples of Porphyridium, F720 was highest even with phycobilin excitation.

To obtain information about the relative contribution of the three components, their shapes were estimated as follows. As shown in Fig. 1, the spectrum of Synechococcus excited at 437 nm consists to a large extent of the F720 band, while in the spectrum of Anacystis excited at 547 nm the contribution of this band is low.

We assume that the shape of the F720 band is the same for both species, but its maximum is shifted to 715 nm in Anacystis. The shapes of the F685 and F695 bands are estimated from experiments with samples in which one dominates the other. Assuming half-width values of 10, 15 and 28.5 nm for the three bands it was possible to calculate other measured fluorescence spectra with a satisfactory approximation, using the percentages given in Table I.

TABLE I

RELATIVE CONTRIBUTION OF THE F685, F695 AND F720 FLUORESCENCE BANDS OF CHLOROPHYLL *a* TO THE CHLOROPHYLL *a* SPECTRUM *in vivo*

The F720 maximum is set at 100. Some samples of Porphyridium showed, with phycobilin excitation, values of F685 and F695 up to 70 and 250.

	Chlorophyll excitation			*Phycobilin excitation*		
	F685	*F695*	*F720*	*F685*	*F695*	*F720*
Anacystis						
4 days	250	225	100	125	100	100
7 days	1250	500	100	625	125	100
Synechococcus	9.5	14	100	18	63	100
Anabaena	7	13	100	12	60	100
Oscillatoria	9	12	100	38	46	100
Nostoc	2.5	7	100	47	73	100
Porphyridium	8	12	100	25	42	100
Porphyra	2.5	4	100	20	34	100

Both F685 and F695 show the chlorophyll *a* type of fluorescence spectrum as measured in organic solution. A similar spectrum is measured *in vivo* with etiolated bean leaves in the first stages of greening[4]. F720, however, is much broader while its shape resembles more that of the bacteriochlorophyll fluorescence spectrum than that of chlorophyll *a*. Table I shows that, with all species measured, light energy absorbed by phycobilins is more readily transferred to the chlorophyll forms responsible for F685 and F695 than it is for the one responsible for to F720 emission.

Since at room temperature phycobilins are primarily transferring energy to System II of photosynthesis, the conclusion of several authors that F685 and F695 receive energy from pigments belonging only to System II seems justified[5–8].

The suggestion, made earlier by the present author, that F695 represents emission by the main C_a680 component, is unlikely, also in view of the following: (1) C_a680 is present in the excitation spectrum of F720 at —130°, when F695 is not yet measurable; (2) heating to 80° may destroy F695, while C_a680 stays in the absorption and the excitation spectrum; (3) the absorption spectrum at —77 °K shows a C_a680 band sharper than the C_a670 band while on the contrary F695 is broader than F685. Also, it is evident from Fig. 1 that the ratios F685/F695 for the Anacystis sample given are not identical in the two curves. Anacystis cells cooled to 77 °K in the dark and illuminated with strong blue light showed, as was measured with Chlorella[9], a decrease in fluorescence intensity which was most pronounced for F695. Therefore the blue light spectrum, in which F695 is lowest, was taken before the green light one, in which such an effect was not observed. Hence this effect cannot be the cause of the difference in ratio F685/F695. As a consequence it is likely that the action spectra of F685 and F695 are not, at least for this sample, identical.

In view of the experiments with Euglena, where a marked C_a695 band is present both in the absorption and fluorescence action spectrum of F720 (at room temperature as well as at 77 °K, *cf.* refs. 11 and 10), the F720 band is supposed to be emitted by a compound C_a695 in general. It then follows that C_a680, which represents probably the bulk of the chlorophyll *a* of System I, transfers its energy to F720 without emitting fluorescence at —196°. In order to account for various bands of accessory pigments and chlorophyll *a* in the action spectra measured with different groups of algae and chloroplasts of higher plants, it is necessary to assume that F720 also receives energy from pigments of System II. In blue-green and red algae, carotenoids do not transfer energy to chlorophyll at room temperature (*cf.* refs. 12, 10 and 1). In the excitation spectrum measured at —196°, however, two bands appear at 475 and about 510 nm, which are clearly seen in the action spectra of phycobilin-free lamellae and chloroplasts of blue-green and red algae. In Anacystis they can only be detected in the 3-day-old culture, though even here they are less marked than with other blue-green algae. This indicates that these bands are absent in the action spectrum of F695 (*cf.* also action spectra given by BERGERON AND OLSON[2]) and only connected to the F720 band. Thus they belong either to a pigment transferring energy to F720 chlorophyll *a*, or to F720 chlorophyll *a* itself. In the first case it is likely that, in view of the considerations made above, they belong to a pigment participating only in System I of photosynthesis.

Investigations are in progress to study the nature of these bands, which, in view of their location, could belong to a carotenoid.

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