BBA 45958

LOW-TEMPERATURE (4–77°K) SPECTROSCOPY OF CHLORELLA; TEMPERATURE DEPENDENCE OF ENERGY TRANSFER EFFICIENCY

F. CHO AND GOVINDJEE*

Department of Botany and Division of Biophysics, Department of Physiology and Biophysics, University of Illinois, Urbana, Ill. 61801 (U.S.A.)

(Received November 10th, 1969)
(Revised manuscript received April 2nd, 1970)

SUMMARY

From the absorption spectra and the excitation spectra of chlorophyll *a* fluorescence measured (4–77°K) at different wavelengths (685–760 nm) and their comparison with the emission spectra published elsewhere[23], we confirm that both the Pigment Systems (I and II) contain chlorophyll *b*, chlorophyll *a* 670 and chlorophyll *a* 678. But System II is relatively enriched in chlorophyll *b* and System I in chlorophyll *a* 685–715. Moreover, our data suggest the following correspondence between absorption and fluorescence bands: chlorophyll *a* 678 is responsible for F687, "Trap II" for F698 and chlorophyll *a* 685–715 for F725.

The efficiencies of energy transfer from chlorophyll *b* to chlorophyll *a* and from chlorophyll *a* 670 to chlorophyll *a* 678 that approach 100 % even at 4°K were found to be independent of temperature (4–295°K). However, transfer efficiencies from chlorophyll *a* to the suggested trap of System II may be temperature dependent.

INTRODUCTION

Upon cooling to 77°K, the fluorescence yield of intact algae (and chloroplasts) increases 5–10-fold[1]; the main emission band at 685 nm (F685) becomes triple peaked (F687, F698 and F725 (refs. 1–5)) and the broad absorption and fluorescence excitation bands at 675 nm are resolved into two peaks at 670 nm and at 678 nm (refs. 6–8). We have extended these spectroscopic measurements to temperatures down to 4°K where the fluorescence yield is about twice that at 77°K. We present absorption spectra of the same sample at 4°K and 77°K.

The three fluorescence bands (F687, F698 and F725) have been ascribed to different forms of chlorophyll *a* (refs. 9–12). Our data suggest that chlorophyll *a* 678 (but not chlorophyll *a* 670) is responsible for F687; chlorophyll *a* 670 emits at 681 nm (F680) when chlorophyll *a* 678 is selectively removed by extraction with acetone–water mixtures[13,14]; the F698 is suggested to originate in the trap of System II (refs.

Abbreviations: We will use F687, F698 and F725 for fluorescence bands at low temperatures even though the exact locations may vary; the prefix "f" will be used to denote the fluorescence intensity at a certain wavelength.

* Please send reprint requests to: GOVINDJEE, 297 Morrill Hall, University of Illinois, Urbana, Ill. 61801, U.S.A.

3, 5, 15); the chlorophyll *a* 685–715 is responsible for the F725 complex band; and the f750–760 are partially related to the vibrational bands of F687 and F698.

The mechanism of energy transfer has recently been discussed by PEARLSTEIN[16] and by ROBINSON[17]. Whether the first-order (αR^{-3}, fast)* or the second-order (αR^{-6}, slow) transfer is a closer approximation for the energy transfer rate in photosynthetic organisms has not yet been settled. Of course, the mechanism of energy transfer can vary among different pigments in the same organism. FÖRSTER[18] suggested that for the R^{-6} case the transfer rate could be temperature dependent but not for the R^{-3} case. This data is not available in the literature[19]. Since transfer rate could be related to transfer efficiency under some conditions (see p. 65–67 in ref. 15; ref. 20), we present the efficiency of energy transfer *in vivo* as a function of temperature.

MATERIALS AND METHODS

Chlorella pyrenoidosa was grown as described earlier[21]. Absorption spectra at low temperatures were measured by an instrument described by CEDERSTRAND *et al.*[22] but with the following modifications (Fig. 1). Incident light from the monochromator (half band width, 7 nm) entered the sample compartment by a fiberglass light-guide. The sample consisted of a thin (approx 0.02 mm) paste of algal cells spread evenly between two glass cover slips, and the sample "sandwich" was clamped with springs between the first light guide and another through which the transmitted and fluorescent light left the sample. The sample compartment, a Dewar flask, was filled with liquid He (for 4°K) through a transfer tube[23]; an outer jacket contained liquid N_2. (For 77°K measurements, liquid N_2 was poured directly into the inner compartment.) The level of liquid He, which was kept above the sample, was detected by a solid-state temperature sensor

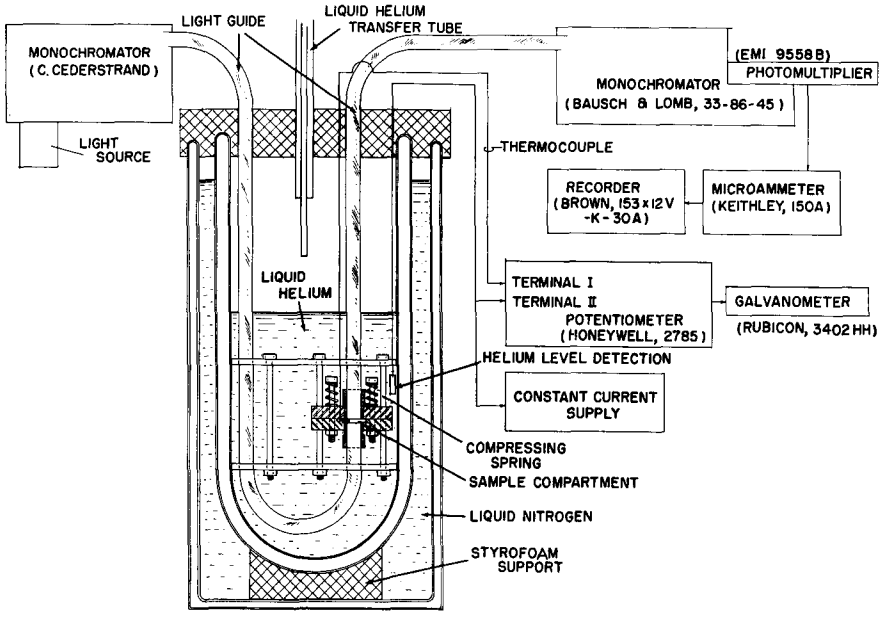


Fig. 1. Block diagram of the apparatus for low-temperature absorption measurements.

* See NOTE ADDED IN PROOF.

(Model CD 5004, Phylatron Corp. Columbus, Ohio). The temperature was measured with a thermocouple (advance No. 34, chromel No. 34) connected to a potentiometer (Model 2765, Honeywell Co., Denver, Colo.) and a null-detecting galvanometer (Model 3402 HH, Rubicon Co., Philadelphia, Pa.); the temperature measurements were calibrated against a platinum thermister. The transmitted and the fluorescent light entered a Bausch and Lomb monochromator (half band width, 6.6 nm) set at the same wavelength as the monochromator which provided the incident light; the fluorescence was thus prevented from hitting the photomultiplier (EMI 9558B) placed at the exit slit of the analyzing monochromator except in the region of absorption and fluorescence overlap. (In some experiments EMI 9558B phototube was replaced by RCA C70007A with no change in results.) The output current of the photomultiplier amplified by a microvoltammeter (Model 150A, Keithley Instruments, Cleveland, Ohio) was registered on a recorder (Model 153 × 12 V-K-30A, Brown Instruments Division, Minneapolia-Honeywell Reg. Co., Philadelphia, Pa.). Both the I_0 (transmitted light intensity of the reference beam using water or bleached cells) and the I (transmitted light intensity using cells as sample), as a function of wavelength, were measured separately. The calculated absorbance was corrected for apparent absorbance at 800 nm.

Fluorescence excitation spectra were measured by a spectrofluorimeter described earlier[24,25] but with proper modifications for measurements at 4°K (ref. 23). The excitation monochromator had half band widths ranging from 2.4 to 6.6 nm, and the analyzing monochromator had a half band width of 6.6 nm. During fluorescence excitation spectra measurements for f725, a colored glass filter C.S. 7-69 was placed between the sample and the analyzing monochromator, but C.S. 2-64 was used for f687 and f698. All the excitation spectra are expressed in fluorescence intensity per incident quanta as a function of wavelength. For further details of the materials and method see ref. 15.

RESULTS AND DISCUSSION

Absorption spectra

Low-temperature (77°K) absorption spectra of photosynthetic organisms in the red region were reported by BUTLER[8], KOK[2] and FREI[6]. They showed that the broad chlorophyll *a* 675-nm band (at room temperature) was resolved into chlorophyll *a* 670 and chlorophyll *a* 680 bands. A new band at 705 nm (chlorophyll *a* 705) was present in several organisms and the authors[2,8] concluded that it was not from P700 (the energy trap of System I).

Fig. 2 shows the complete absorption spectra of thin films of *C. pyrenoidosa* from 400 to 720 nm at 4 and 77°K. The broad 675-nm absorption band (at room temperature) is resolved into two bands (at 670 and 677.5 nm) both at 77 and 4°K. The 77°K spectrum in the red region agrees qualitatively with those presented by others[2,6,8]. The red band of chlorophyll *b* is observed at 649.5 nm. The bands in the blue region cannot be easily analyzed due to the overlap of bands due to chlorophyll *a*, chlorophyll *b* and the carotenoids[12,26,27]. They are tentatively assigned to: chlorophyll *a plus* carotenoids (440 nm), carotenoids (463–467 nm and 491 nm) and chlorophyll *b* (476.5 nm). Minor bands around 625, 595, 575 and 540 nm are due to chlorophyll *a* and chlorophyll *b*.

The absorption spectra at low temperatures provide two important pieces of information (1) the variations of absorbance are very small when Chlorella is warmed

from 4 to 77°K; this information is needed for the calculation of the efficiency of energy transfer; (2) the absorption bands observed at these temperatures have been shown to be present at room temperature where they are poorly resolved[22,27].

Excitation spectra

The efficiency of energy transfer from the accessory pigments to chlorophyll *a* is calculated from the excitation spectra of chlorophyll *a* fluorescence and the absorption spectra of the system (see APPENDIX). At room temperature, the efficiency of energy transfer from chlorophyll *b* to chlorophyll *a* is almost 100 % in Chlorella; however, the transfer efficiency from the carotenoids to chlorophyll *a* is only 40–50 % (ref. 28).

At 77°K, the excitation spectrum of Chlorella shows bands at 440, 470, 490–492, 580, 625, 651, 672 and 682 nm (ref. 7). BUTLER AND BAKER[29] showed an additional band at 705 nm in the supernatant of sonicated Chlorella; this band has also been observed in spinach chloroplasts[1] and seems to be associated mainly with the Pigment System I (refs. 24, 30 and 31).

In the present investigation, the fluorescence excitation spectra of Chlorella were measured at different temperatures down to 4°K. The lower temperatures (4–77°K) used provided good resolution, higher yields and were not influenced by the

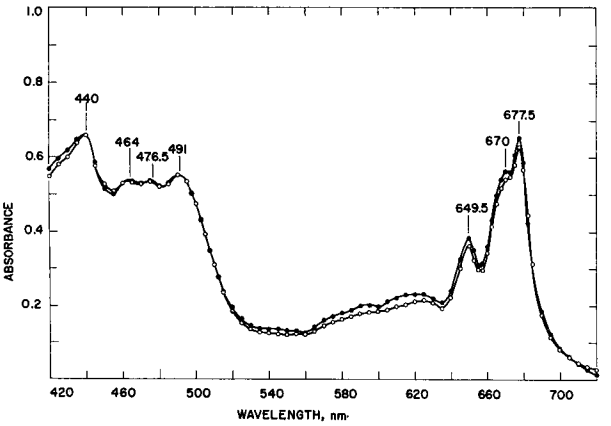


Fig. 2. Absorption spectra of C. *pyrenoidosa* measured at 4°K (●—●) and 77°K (○—○).

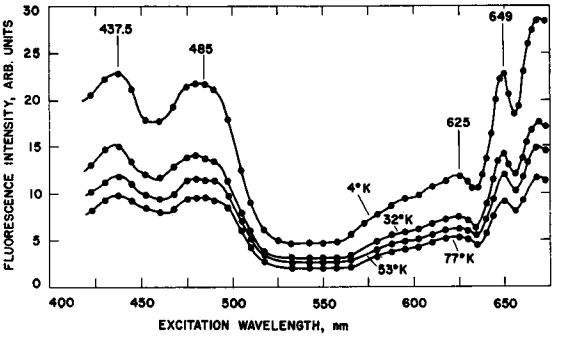


Fig. 3. Fluorescence excitation spectra measured at 685 nm (f685) of *C. pyrenoidosa* in the 4–77°K range (half band width of excitation = 6.6 nm).

phase transitions of the ice crystals[40]. From these results and from the emission spectra[23] the temperature dependence of the energy transfer efficiency among different molecules is estimated (see APPENDIX), and evidence is found suggesting that the F687 is emitted from chlorophyll *a* 678 instead of chlorophyll *a* 670.

Fig. 3 shows the excitation spectra of Chlorella for f685 at four different temperatures (4, 32, 53 and 77°K). The following bands are observed: at 438 nm (mainly chlorophyll *a*), 485 nm (chlorophyll *b* and carotenoids), 625 nm (chlorophyll *a*), 649 nm (chlorophyll *b*), 670 nm (chlorophyll *a*) and some minor chlorophyll bands (525 to 620 nm). In the absorption spectra (Fig. 2) there are two bands at 476.5 and 491 nm in this region, but in the excitation spectra (Fig. 3) these bands are not clearly resolved, and a broad 485-nm band is observed; this could partly be due to the inefficient participation of certain carotenoids in the energy transfer to chlorophyll *a* (*cf.* ref. 12).

The ratio of the fluorescence intensity excited by 438 nm (chlorophyll *a*) to that by 476 nm (chlorophyll *b*) in the excitation spectrum for f685 at 4°K is about 1.0. This low ratio (*cf.* with Fig. 2) suggests that the relative participation of chlorophyll *b* for f685 is high, and thus, it is mainly from Pigment System II. Furthermore, this ratio remains constant with the change in temperature (4–77°K). Calculations based on the method described in APPENDIX showed that the efficiency of energy transfer from chlorophyll *b* to chlorophyll *a* 670, and from chlorophyll *a* 670 to chlorophyll *a* 678 (in both the Pigment Systems I and II) remained constant with temperature: the efficiency of energy transfer was 95–100%.

The yield of fluorescence at 685 nm, as excited by 437.5 nm, increased by a factor of 2.3 from 77 to 4°K. (It is to be noted that both absorption and fluorescence were measured with the same band width of excitation.)

Fig. 4 shows the excitation spectra (in the 635–690 nm range) of f685, f690 and f698, at 77°K, in Chlorella. In this experiment, the half band width of the exciting and the analyzing monochromators was only 2.46 nm. With such narrow band widths, the excitation could be scanned to within 5–6 nm of the observation wavelength without noticeable deflection caused by the leakage of light. It was possible to go over the

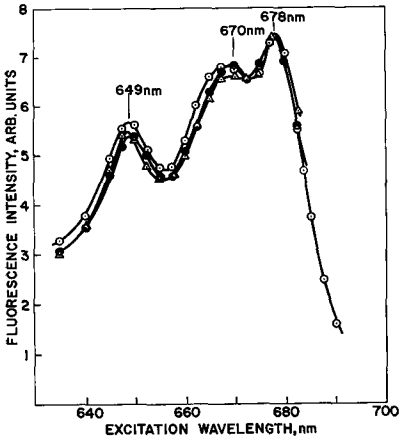


Fig. 4. Fluorescence excitation spectra for f687, f690 and f697 of *C. pyrenoidosa* measured at 77°K. The half band width for both the exciting and analyzing monochromators was 2.64 nm. The cut-off filter C.S. 2-64 was used before the analyzing monochromator. ●—●, f687; △—△, f690; and ⊙—⊙, f698.

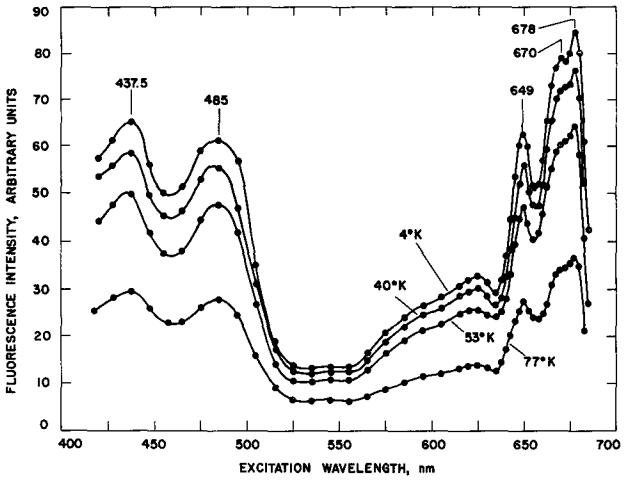
chlorophyll *a* 670 and chlorophyll *a* 678 bands for all the measurements, but the curve could be extended beyond the main emission band only for f698. All three spectra show bands at 649 nm (chlorophyll *b*), 670 nm (chlorophyll *a* 670) and 678 nm (chlorophyll *a* 678) and their ratios are similar.

From the above data and from the emission spectra at low temperatures[23], two important conclusions are drawn: (1) The pigment system that produces fluorescence at 685, 690 and 698 nm must be the same because of the close identity of the excitation spectra observed here and also because of similar fluorescence transient kinetics at low temperatures[15,32]. The observed ratios of the chlorophyll *b* (649 nm) to the chlorophyll *a* (670, 678 nm) bands are indicative of their System II character (see refs. 1, 30 and 33). (2) The F687 is emitted when chlorophyll *a* 678 is excited. This result indicates that chlorophyll *a* 678 emits at 687 nm; chlorophyll *a* 670 cannot emit at 687 nm because it would mean an uphill energy transfer from chlorophyll *a* 678 to chlorophyll *a* 670 to produce this fluorescence. Our attempts to find evidence of an uphill energy transfer (see ref. 34) in Chlorella have failed. The Stoke's shift in chlorophyll systems is found to range from 5 to 10 nm. The Stoke's shift of 9 nm for chlorophyll *a* 678–687 is, therefore, reasonable. This assignment is further supported by the occurrence of the emission at 681 nm (due to chlorophyll *a* 670) when chlorophyll *a* 678 is selectively removed[13,14].

Since the chlorophyll *a* 678 is responsible for F687, it cannot be responsible for F698. It has been suggested that F698 is an emission from the energy trap of System II (refs. 3, 5 and 10). It is sensitized preferentially by System II and the System II particles are enriched in it[24,30]. If so, this species must be present in very low concentrations and difficult to observe in the absorption spectra and fluorescence excitation spectra of F698. It has been shown[40] that the F698 is sensitive to the form and composition of the aqueous environment (change in ice phase and treatment with 10% dimethylsulfoxide); this is consistent with the hypothesis that it may be from the trap. Recently DONZE AND DUYSENS[32] and MURATA[35], on the basis of low-temperature fluorescence transients, have also suggested that the F698 may be from the Trap II. Furthermore, one can predict, on the basis of an approximate Stoke's shift of 10 nm, an absorption band at 688 nm for this trap. Recently DÖRING *et al.*[36] have discovered an absorbance change due to the energy trap of System II in the 682–690-nm range (also see ref. 41).

Fig. 5 shows the excitation spectra for f698 of *C. pyrenoidosa* measured at 4, 40, 53 and 77°K. These spectra show the same bands (at 437.5, 485, 649, 670 and 678 nm) as observed for f685. Also, the ratios of the bands at 437.5 nm (chlorophyll *a*) to that at 485 nm (chlorophyll *b* and carotenoids) (1.1) and at 670 nm (chlorophyll *a*) to that at 649 nm (chlorophyll *b*) (1.25) are approximately the same as for f685. These results confirm that the pigment system producing f698 is the same as that for f685 (System II).

Examination of the emission spectra of Chlorella as a function of temperature[23] shows that the half band width of F698 and F687 and their relative intensities are not drastically different except at 4°K. Moreover we have used narrow band widths for the observation (6.6 nm) of f685 and f698. Under these conditions, if there were differences in the composition of the pigment systems that produce F687 and F698, we should have been able to see them because at 687 and at 698 nm there are preferentially high contributions by F687 and F698, respectively (at least in the 77–26°K range). Thus, we retain the conclusion that the pigment system that produces F698 is the same as that for F687.



Fih. 5. Fluorescence excitation spectra for f698 of *C. pyrenoidosa* in the 4–77°K range.

The ratios of the bands of chlorophyll *a* to those of chlorophyll *b* are independent of temperature (4–77°K range). Since the absorption spectra did not change in this temperature range, these data suggest that the efficiency of energy transfer from chlorophyll *b* to chlorophyll *a* remains independent of temperature. Calculations based on the method described in APPENDIX showed that the efficiency of energy transfer from chlorophyll *b* to chlorophyll *a* 670 and from chlorophyll *a* 670 to chlorophyll *a* 678 (in both the Pigment Systems I and II) was about 95–100 % and remained constant with temperature.

The increase in the fluorescence intensity at f698 in going from 40 to 4°K was less than that for f685. This phenomenon was also shown by the dramatic decrease of the fluorescence ratio of f698/f687 in the 4–26°K temperature range (see ref. 23, and Figs. 3 and 5, this paper). We raise the question whether it means that the efficiency of energy transfer from chlorophyll *a* 678 to the chlorophyll *a* form that produces F698 (the Trap II) decreases at very low temperature (4°K) — and the "slow" transfer mechanism operates in this case. No definite answer can be given at this time.

As noted for f685 the yield of fluorescence at 698 nm, excited by 437.5 nm, also increased by a factor of 2.2 from 77 to 4°K.

Fig. 6 shows the excitation spectra of fluorescence for f725, as a function of temperature; the room temperature excitation spectrum (open circles) is given for comparison. In the latter spectrum, the usual peaks at 675 nm (chlorophyll *a*), 650 nm (chlorophyll *b*), 480 nm (chlorophyll *b* and carotenoids) and 437.5 nm (chlorophyll *a* and carotenoids) are observed. By comparing the absorption and the excitation spectra, one finds a lower fluorescence yield in the blue region as expected from the lower efficiency of energy transfer from the carotenoids to chlorophyll *a* (ref. 28).

Upon cooling to 4°K, the broad 480-nm band is resolved into two bands at 477.5 nm (chlorophyll *b* and carotenoids) and at 491 nm (carotenoids), and the broad 675-nm band is resolved into bands at 672.5 nm (chlorophyll *a*) and at 679 nm (chlorophyll *a*); other bands are at 440 nm (chlorophyll *a* and carotenoids) and at 649 nm (chlorophyll *b*) and a shoulder around 705 nm (chlorophyll *a* 705). These band locations are in good agreement with the excitation spectra measured at 77°K by GOEDHEER[7]

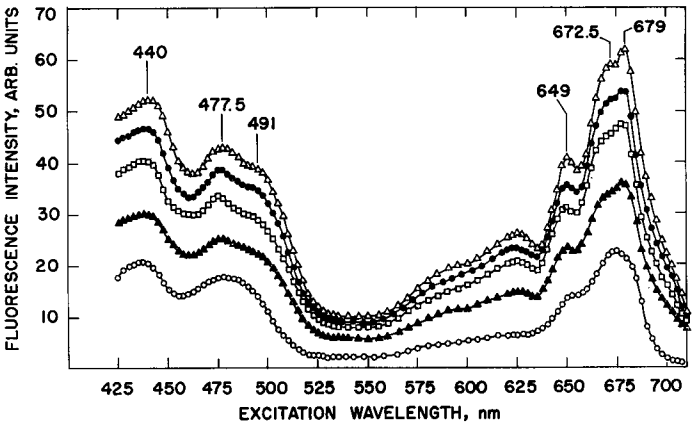


Fig. 6. Fluorescence excitation spectra for f725 of *C. pyrenoidosa* measured at 4°K (△—△), 37°K (●—●), 58°K (□—□), 77°K (▲—▲) and 295°K (○—○).

except that the 477.5-nm band is at 471 nm in his curves; the 477.5-nm band moves to 471 nm when we remove the cut-off filter (C.S. 7-69) placed before the analyzing monochromator. The presence of a band at 491 nm in the excitation spectra for f725, and not in that of f698 and f685, suggests that certain carotenoids are effective in transferring energy only to chlorophyll *a* forms (in System I) that fluoresce at 725 nm. GOEDHEER[12] has recently shown excitation bands at 470 nm and at 495 nm in chlorophyll *b*-less *Tribonema equale* and in other systems; he ascribed these bands to *β*-carotene.

The ratio of the 440-nm to 477.5-nm bands is 1.24 for f725; this is higher than for both f698 and f685 confirming that the pigment system producing f725 is different from that producing f698 (and f685); for f725, there is greater contribution from chlorophyll *a* (System I). Complications due to light absorption by the carotenoids do not allow us to make precise conclusions in the blue region of the spectrum. However, the ratio (1.45) of the 672.5-nm (chlorophyll *a*) to 649-nm (chlorophyll *b*) band is also higher for f725 than for f698 (and f685) confirming that there is a greater contribution of chlorophyll *a* (System I) to f725. The conclusion that f725 is preferentially from System I is further supported by the reduction in the ratio of variable to constant fluorescence in the fluorescence transients at low temperature[37]; it is 60% from System I, and 40% from System II (ref. 15).

We note that the chlorophyll *a* 670 band in the f725 excitation spectra appears to be shifted to red by about 2.5 nm (in some experiments) from that in the spectra of f698 and f685; also, the chlorophyll *a* 680 band is at 679 nm in the f725 spectra, and at 678 nm in the f698 and f685 spectra. We do not believe that these shifts are significant. However, if proven significant, these may be caused either by a distortion due to the presence of additional bands (see below) or be real differences in the location of the red bands of chlorophyll *a* in System I and System II.

The ratios of bands at 440 nm to those at 477.5 nm, and of 649 nm to 672.5 nm, are independent of temperature from 77 to 4°K. This suggests that the efficiency of energy transfer from chlorophyll *b* to chlorophyll *a* (in System I also) is independent of temperature. This was confirmed by the calculations (based on the method in APPEN-

DIX) that the efficiency of energy transfer from chlorophyll *b* to chlorophyll *a* 670 and from chlorophyll *a* 670 to chlorophyll *a* 678 was about 95–100%, and it remained constant with temperature.

The temperature independence of the transfer efficiency from chlorophyll *b* to chlorophyll *a*, and from chlorophyll *a* 670 to chlorophyll *a* 678 (in both pigment systems, see Figs. 3, 5 and 6) requires an explanation. If the number of transfers among the homogeneous molecules (*i.e.* chlorophyll *b* or chlorophyll *a* 670) is large (for example, 10^4), then the slow transfer mechanism (1–100 psec) within each one of them is *not* possible because it would take 0.01–1 μsec before energy is dissipated, but we know that this is not the case since the measured life time of the excited state *in vivo* is approx. 1 nsec. However, if the transfer number is very small, the irreversible transfer from chlorophyll *b* to chlorophyll *a*, and from chlorophyll *a* 670 to chlorophyll *a* 678 will lead to the observed result regardless of the transfer rate (fast (0.01–1 psec) or slow (1–100 psec)) among the homogeneous molecules because the transfer rate from chlorophyll *b* to chlorophyll *a* and from chlorophyll *a* 670 to chlorophyll *a* 678 would still be faster than the dissipation of energy from chlorophyll *a*.

The shoulder around 705 nm in the excitation spectra for f725 is not very obvious. Thus, a detailed analysis was made. Fig. 7 shows detailed excitation spectra for fluorescence measured at 698 and at 725 nm and normalized at 664 nm. (The normalization was made arbitrarily at 664 nm to show differences both in the chlorophyll

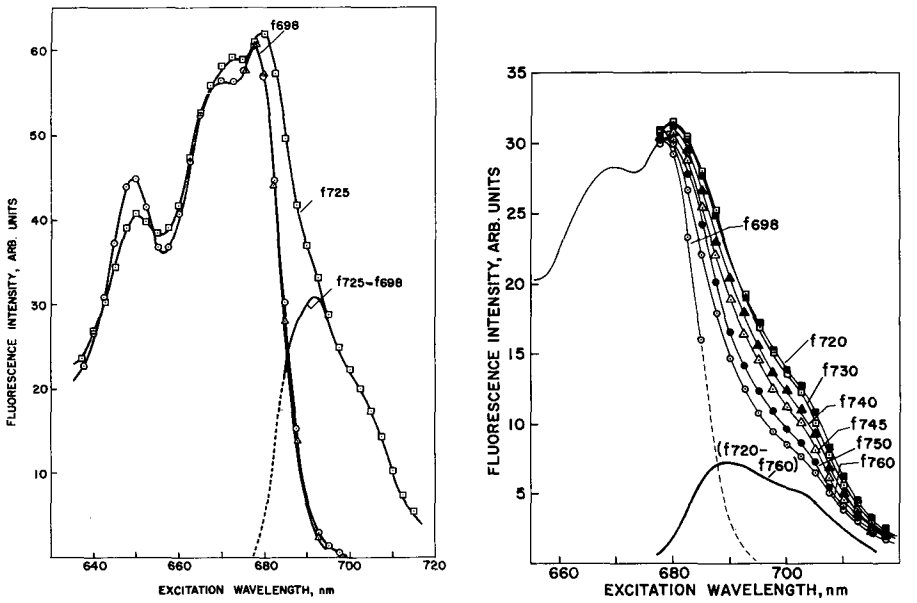


Fig. 7. Fluorescence excitation spectra for f725 and f698 of *C. pyrenoidosa* measured at 4°K. The two spectra were arbitrarily normalized at 664 nm. The excitation spectrum for f698 beyond 687 nm was estimated (a) by correction for the "light-leak" (△—△), and (b) by Gaussian extrapolation (⊙—⊙). A portion of the difference spectrum (f725–f698) is shown by dotted line.

Fig. 8. Fluorescence excitation spectra of *C. pyrenoidosa* measured at 4°K. Fluorescence was measured at 698 nm (f698), 720 nm (f720), 730 nm (f730), 740 nm (f740), 745 nm (f745), 750 nm (f750) and 760 nm (f760). The continuous graph in the 660–680-nm region is for f698, and that in the 680–720-nm region is for the difference spectrum (f720–f760).

b and chlorophyll *a* regions.) True fluorescence for f698 beyond 687 nm was obtained by subtracting the "light leak" from the measured values. Another method to estimate the excitation spectrum beyond 687 nm was to assume that the F698 curve follows a Gaussian distribution. Both the methods gave the same result. The difference excitation spectrum (f725 *minus* f698) shows a complex structure having a peak at about 690 nm, and a shoulder around 705 nm. It is obvious that this difference spectrum is composed of several components. The complexity of the excitation spectra in the 680–720 nm range is further shown in Fig. 8. In this figure, the excitation spectra for f698, f720, f730, f740, f745, f750 and f760, and the difference excitation spectrum (f720–f760) is shown. This difference spectrum is also complex and confirms that there are several long wave chlorophyll *a* forms (680–710 nm) responsible for fluorescence in the 720–760 nm range. Moreover, it shows clearly that the long wave forms of chlorophyll *a* (680–710 nm) preferentially excite fluorescence at 720 nm than at 760 nm. The lower contribution of the long wave chlorophyll *a* forms in producing fluorescence at 760 nm is due to the presence of satellite fluorescence bands of the short wave chlorophyll *a* forms (F687, F698).

A comparison of Figs. 3–6 (also see emission spectra) suggests the presence of chlorophyll *b* and both chlorophyll *a* 670 and chlorophyll *a* 678 in both the pigment systems (I and II) because both of these bands are present in the excitation spectra for f725 (60% in System I), f698 (mainly System II) and f685 (mainly System II). However, Figs. 7 and 8 show that the long wave forms of chlorophyll *a* (680–710 nm) preferentially excite f720, *i.e.* these chlorophyll *a* forms are present mainly in System I. (For the assignment of f720 to a long wave form of chlorophyll *a*, also see DAS AND GOVINDJEE[38].) Our analysis is in qualitative agreement with the action spectra of the Pigment Systems I and II (refs. 22 and 39), and the analysis of the separated pigment systems[30,31].

APPENDIX

The temperature dependence of the energy transfer efficiency of the two pigment systems was determined by comparing the sensitized fluorescence measured at various temperatures. The two Pigment Systems (I and II) were considered separately for the convenience of calculations. The derivation of the method for the energy transfer from chlorophyll *a* 670 to chlorophyll *a* 678 in System II is shown as follows.

Let E_{678}^{687} and E_{670}^{687} be the intensities of the System II fluorescence at 687 nm (F687), excited by 678 and 670 nm (subscript), A_{678} and A_{670} be the number of quanta absorbed in System II at 678 and 670 nm, Φ_{678}^{687} be the System II fluorescence efficiency of F687 (superscript) when excited at 678 nm (subscript) and $R_{670\rightarrow 678}$ be the transfer efficiency (percent transferred) from chlorophyll *a* (670) to chlorophyll *a* (678) in System II. Then:

$$E_{678}^{687} = A_{678}\Phi_{678}^{687} \tag{1}$$

$$E_{670}^{687} = A_{670} \cdot R_{670\rightarrow 678} \cdot \Phi_{678}^{687} \tag{2}$$

The ratio of Eqn. 1 and 2 gives:

$$R_{670\rightarrow 678} = \frac{E^{687}_{670}}{E^{687}_{678}} \cdot \frac{A_{678}}{A_{670}} \qquad (3)$$

The right hand side of Eqn. 3 was determined from the intensity of the bands at 670 and 678 nm of F687 (see Fig. 4), and from the ratio of the System II absorption bands at 678 and 670 nm which were determined from the absorption data of Fig. 2 and from the action spectra of photosynthesis of the two pigment systems[22,39]. The temperature dependence of System II energy transfer efficiency from chlorophyll *a* (670) to chlorophyll *a* (678) was determined by comparing $R_{670\rightarrow 678}$ measured at different temperatures. Other transfers were calculated by a similar method.

NOTE ADDED IN PROOF (Received June 12th, 1970).

HOCH AND KNOX[40] have recently argued that the idea of 'R^{-3} transfer' is in itself a volatile concept.

ACKNOWLEDGMENTS

Thanks are due to the National Science Foundation for support during the course of the present investigation.

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