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Emission spectra of Chlorella at very low temperatures (—269° to —196°)

BRODY[1] discovered a new band at 720 mμ (F720) in Chlorella at —196°. Recently several investigators[2–9] have shown that there is an additional band at about 696 mμ (F696). Earlier data[8–10] suggest that the intensities of the 3 fluorescence bands —F685, F696 and F720—change at different rates with change in temperature (—196° to +20°). We report here the emission spectra of *Chlorella pyrenoidosa* from —269° to —196° upon excitation by blue light (485 ± 5 mμ).

Chlorella pyrenoidosa was grown in completely inorganic medium for 6 days (see ref. 11 for growth conditions). A folded piece of cheese-cloth was placed at the bottom of a 32-cm tall Dewar flask having an optically clear and a rounded glass bottom. A very thin suspension of Chlorella was dropped onto the cheesecloth by a syringe. A plunger made of teflon was used to press the material to the bottom surface of the Dewar. The sample was cooled to liquid helium temperature (—269°) by transferring liquid helium to the bottom of the Dewar flask at a fast rate, by means of an adjustable helium transfer tube made of stainless steel. Pressurized helium gas (approx. 5 lb·inch^{-2}) was forced into the helium container to achieve the transfer.

The temperature was measured with a thermocouple inserted in the plunger so that its junction rested in the sample. The thermocouple, made of chromel No. 34 and advance No. 34, was calibrated by a platinum thermometer. The temperature was recorded on a Varian G-10 recorder. A temperature of —269° could be maintained for at least 5 min at a time. This was long enough to plot an emission spectrum. As the liquid helium boiled off, the temperature increased, and emission spectra could be measured as a function of temperature (from —269° to room temperature).

Emission spectra were measured by an automatic spectrofluorometer[10]. The measuring slits had a band width of 3.3 mμ. A Corning C.S. 2–58 filter was placed before the analyzing monochromator. Fluorescence was collected from the same surface that received the excitation light. The emission spectra (recorded on a Brown recorder) were corrected for the sensitivity of the photomultiplier (EMI9558B) and for the variations in the efficiency of the analyzing monochromator as a function of wavelength. The excitation light (485 ± 5 mμ) was obtained from a tungsten (6 V, 18 A) lamp, and filtered through a large Bausch and Lomb monochromator.

Fig. 1 shows the emission spectra measured in the 680–720-mμ range for several temperatures (—269°, —247°, —233°, —218°, and —196°). Upon warming the sample (—247° to —196°), a shift from 695 mμ to 699 mμ in the location of the peak of the "F697.5" band is noticeable. As the temperature decreases from —196° to —269°, the fluorescence intensity increases steadily. The total intensity at —269° is about 2 times that at —196°. The profile of the fluorescence spectrum at —196° shows clearly the F689, F697.5 and F725 bands; at —269°, the F689 appears as a very sharp band and it dominates both F697.5 and F725; the F697.5 shows only as a shoulder at —269°.

The most striking thing observed here is the almost parallel decrease in intensity of the F725 and F697 bands as the Chlorella cells are warmed, from —269° to —196°, whereas the F689 decreases in a different way. The F725/F689 ratio increases from 0.42 at —269° to about 0.84 at —234°, whereas the F697.5/F689 ratio grows from

0.48 (—269°) to 0.94 (—235°); however, the ratio F697.5/F725 remains almost constant (1.2–1.1) in the same temperature change. As a working hypothesis, one may suggest that the F725 and F697.5 are from chlorophyll *a* forms that are performing similar functions so that changes in temperature affect their fluorescence in a similar way. The F696 and F730 were shown[8,9] to decrease (although at different rates) when the temperature was raised from —196° to —100°, whereas the F685 appeared to remain almost constant.

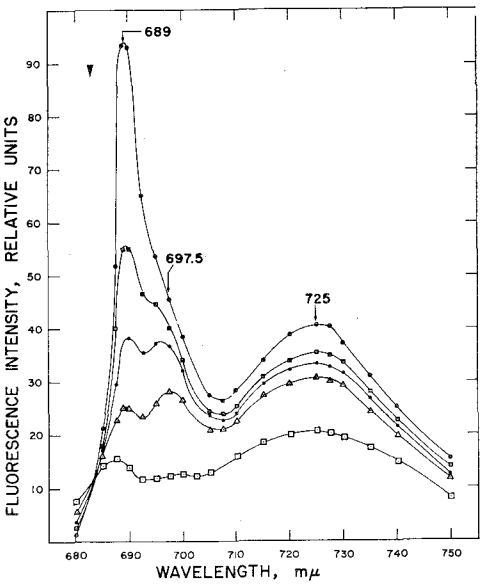

Fig. 1. Emission spectra of *Chlorella pyrenoidosa* as a function of temperature. ⊙—⊙, —269°; ⊡—⊡, —247°; ●—●, —233°; △—△, —218°; ⊡—⊡, —196°.

ROBINSON[12] pointed out that a change occurs in the relative "depth" of the "trap" and the "bulk" pigments, as the temperature is varied in aggregates of certain dyes. As the temperature is decreased, the "trap fluorescence" increases and the "bulk fluorescence" decreases. Whether such a simple conclusion is valid for the complex pigment system in chloroplasts is not yet clear.

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