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ACTION AND EMISSION SPECTRA OF THE LUMINESCENCE OF GREEN PLANT MATERIALS

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Abstract

The action and emission spectra of the delayed light emission from Chlorella, Nostoc, and spinach chloroplasts have been measured. The action spectra for Chlorella and for spinach chloroplasts are quite similar to the absorption spectra of these materials. The action spectrum for Nostoc, on the other hand, shows a relatively low activity for chlorophyll and carotenoids and a high activity for phycocyanin. The emission spectra of these materials demonstrates that the luminescence is the result of a transition between the first excited singlet state and the ground state of chlorophyll. Low-temperature studies suggest that the triplet state of chlorophyll is not involved at all in the luminescence of spinach chloroplasts. There is some indication that part of the light emitted from Nostoc is due to a phycocyanin transition.

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A study of the spectral characteristics of the delayed light emission of green plant materials has been undertaken. The apparatus used was identical to that reported earlier[2] except that a longer time constant (0.1 second) for the photomultiplier amplifier circuit was used here in order to integrate noise and to permit operation at a higher gain. For the measurement of the action spectra, a Bausch and Lomb 500-mm focal-length grating monochromator (No. 33-86-45) was placed between the exciting light (General Electric FT-503 flashtube) and the sample. The emission spectra were measured by placing the monochromator between the sample and the detector (Dumont K-1292 photomultiplier). The intensity of the emitted light approximately 0.2 second after excitation by the flash was measured at 10-mμ intervals of either exciting light (action spectra) or emitted light (emission spectra). Two curves were drawn through two sets of alternating points taken 20 mμ apart. The final curve was drawn between the two curves obtained in this way. The slit width of the monochromator corresponded to a band pass of about 20 mμ. A correction for the relative number of quanta in each wave length of exciting light was applied in the action-spectra measurements, and the emission spectra were corrected for the sensitivity of the photomultiplier at the various wave lengths from data supplied by the manufacturer of each of these instruments. All curves, except where otherwise stated, were measured with very thin films of material.

The results of these measurements are shown in Fig. 1. The action spectra for the delayed light emission by Chlorella and by spinach chloroplasts are quite similar to the absorption spectra of these materials, and show both the chlorophyll and the carotenoid bands. Nostoc, on the other hand, has an action spectrum very much different from its absorption spectrum. Both the carotenoids and the chlorophyll are apparently very low in activity. The action spectrum for Chlorella is the same as that obtained earlier by Strehler and Arnold,[3] while that for Nostoc resembles the curve for Synechococcus,[4] another blue-green alga.

The room-temperature emission spectra of the delayed light obtained from thin films of Chlorella and of spinach chloroplasts are essentially identical. A curve for Chlorella has been obtained earlier by Arnold and Davidson.[5] For

*The work described in this paper was sponsored by the United States Atomic Energy Commission.

[1] National Science Foundation Postdoctoral Fellow, 1956-1957.

[2] G. Tollin and M. Calvin, Proc. Nat. Acad. Sci. U.S. 43, 895 (1957).

[3] B. L. Strehler and W. Arnold, J. Gen Physiol. 34, 809 (1951).

[4] W. Arnold and J. Thompson, J. Gen Physiol. 39, 311 (1956).

[5] W. Arnold and J. B. Davidson, J. Gen Physiol. 37, 677 (1954).

purposes of comparison, a typical fluorescence spectrum of Chlorella[6] has been plotted in Fig. 1. The two types of emission are seen to have similar spectra. The fact that the low intensity of the luminescence necessitated the use of rather wide slits in the monochromator accounts for the somewhat broadened shape of the delayed-light-emission spectra as compared with the fluorescence spectrum. Thus, the delayed light emission of both Chlorella[5] and spinach chloroplasts is the result of a transition between the first excited singlet state and the ground state of chlorophyll. In an earlier publication it was tentatively suggested, on the basis of crude measurements with filters on thick films of material, that the luminescence of spinach chloroplasts was the result of a triplet state-to-ground state emission of chlorophyll.[2] This is now recognized as having been due largely to the self-absorption distortion of the emission spectrum (see below).

Emission spectra for thick films of spinach chloroplasts at three temperatures are also plotted in Fig. 1. Thick films were used in order to compensate for the low intensity of the low-temperature emissions.[2] The markedly different shape of these spectra, as compared with the thin film spectra, is due to self-absorption. However, it is apparent that the curves at all three temperatures are identical in shape, thus suggesting that they are the result of the same electronic transition. This temperature independence indicates that the triplet state of chlorophyll is not involved at all in the delayed light emission of spinach chloroplasts.

The delayed light emission of Nostoc is very much weaker than that of either Chlorella or spinach chloroplasts, therefore necessitating the use of thick films of material. In view of this, it would be expected that, if the emission were due solely to chlorophyll, the spectrum would be similar to those obtained with thick films of chloroplasts. However, it is not. This suggests that a significant portion of the emitted light originates in phycocyanin (which has a fluorescence peak at about 660 mμ)[7] and is self-absorbed in the thick layer. The Nostoc emission spectrum also resembles that obtained for Synechococcus.[4]

The action spectra for Chlorella and for spinach chloroplasts demonstrate that electronic excitation energy may be transferred, with some degree of efficiency, from carotenoid to chlorophyll in these materials. On the other hand, transfer from carotenoid to either chlorophyll or phycocyanin proceeds with much lower efficiency in Nostoc. Similar conclusions have been drawn for Chlorella[8] and for Oscillatoria,[9] another blue-green alga, through studies of the photosynthetic and fluorescence action spectra of these organisms.

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[6] E. I. Rabinowitch, Photosynthesis and Related Processes (Interscience, New York, 1956), Vol. II, part 2, p. 1875.

[7] op. cit., p. 1876

[8] op. cit., part 1, pp. 1149-1152.

[9] op. cit., part 2, pp. 1876-1877.

Figure Caption

Fig. 1. Action and emission spectra of the delayed light emission of Chlorella, Nostoc, and spinach chloroplasts. The band width of the monochromator was ~20 mμ. The relative heights of all the curves are roughly comparable.

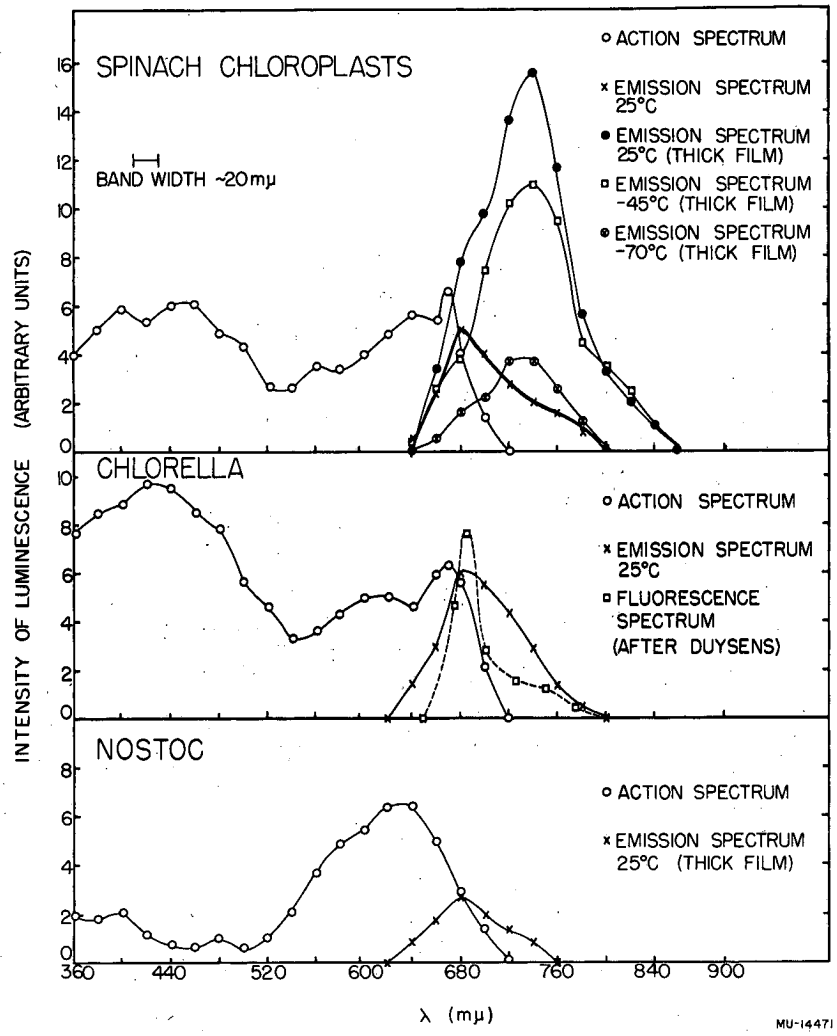
SPINACH CHLOROPLASTS
BAND WIDTH ~20mμ
ACTION SPECTRUM
EMISSION SPECTRUM 25°C
EMISSION SPECTRUM 25°C (THICK FILM)
EMISSION SPECTRUM -45°C (THICK FILM)
EMISSION SPECTRUM -70°C (THICK FILM)
CHLORELLA
ACTION SPECTRUM
EMISSION SPECTRUM 25°C
FLUORESCENCE SPECTRUM (AFTER DUYSENS)
NOSTOC
ACTION SPECTRUM
EMISSION SPECTRUM 25°C (THICK FILM)
INTENSITY OF LUMINESCENCE (ARBITRARY UNITS)
λ (mμ)
360
440
520
600
680
760
840
900
MU-14471

Fig. 1.