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FLUORESCENCE STUDIES ON DEUTERATED *CHLORELLA VULGARIS*

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SUMMARY

Chlorella vulgaris grown in 99.8% heavy water show absorption spectra similar to the absorption spectra shown by those grown in ordinary water; there appears to be a minor shift of about 1 mμ towards shorter wavelengths in the blue and the red absorption bands, and a slight increase in chlorophyll *b* to *a* and carotenoids to chlorophyll *a* ratios in the heavy-water cells. Fluorescence (emission) spectra of deuterated Chlorella show an emission peak which is shifted about 2.0 mμ towards shorter wavelengths. In addition, the ratio of fluorescence at the main peak to fluorescence at 730 mμ is lower in the heavy-water cells, suggesting that they might have more of chlorophyll a_1 fluorescence. The yield of fluorescence is about 20–30% higher in deuterated Chlorella (measured at the emission peak). The excitation spectra of fluorescence show that the energy transfer from chlorophyll *b* to chlorophyll *a* 670 is almost 100% in both deuterated and ordinary hydrogen cells.

INTRODUCTION

It is now well known that Chlorella and many other microorganisms can undergo sustained growth in ^2H_2O (refs. 1, 8). Although some studies of photosynthesis in deuterated organisms have been made[2], many aspects of photosynthetic behavior in deuterated organisms remain to be explored. The current picture of photosynthesis is quite complex, with much of the detailed description unknown. The essential features of the photosynthetic process must be present in ^2H_2O-grown algae. However, the replacement of H by ^2H in all the chemical components of the organism may modify many processes in the algae.

The study of the absorption and fluorescence properties of H_2O-grown and ^2H_2O-grown algae, reported in the present article, was undertaken to compare the light reactions in the two organisms. From a comparison between the absorption properties of the respective pigments in solution[3], it seems that the energy levels and transition probabilities are very nearly the same in the deuterated pigments as in the ordinary pigments. From the fluorescence properties, as observed here, it is concluded that the energy transfer between pigments *in vivo*, as also the percentage of quanta channeled into performing chemical reactions, are very similar in ^2H- and H-algae.

In a parallel study, the influence of the replacement of H by ^2H on the reaction rates and the two pigment systems has been deduced from measurement of the quantum yield of photosynthesis and the Emerson enhancement effect. The deuterated Chlorella show the usual "red drop" and the Emerson effect[4].

MATERIALS AND METHODS

Chlorella vulgaris was grown in a ^2H-labeled medium as described by DABOLL *et al.*[1]. The absorbance was measured by a Bausch and Lomb spectrophotometer (Spectronic 505) equipped with an integrating sphere. The H_2O-grown and ^2H_2O-grown cultures were adjusted to give an absorbance of 0.03 for 0.1-cm path length (the path length of exciting light in our fluorescence instrument) at 680 mμ. Emission and excitation spectra of fluorescence were measured with an automatic spectrofluorimeter[5,6,9]. The emission was measured from the illuminated surface. All the emission spectra have been corrected for the variations in the sensitivity of the photomultiplier and the variations in efficiency of the monochromator with wavelength and the excitation spectra for the incident quanta.

RESULTS AND DISCUSSION

Absorption spectra

The ^2H_2O-grown cells appear to contain a slightly greater amount of chlorophyll *b* and carotenoids than H_2O-grown cells. The differences are of the order of 4–10 %.

Fig. 1 shows the absorption spectra for H- and ^2H-*C. vulgaris*. The two curves have been normalized at 674 mμ; the ^2H_2O-curve was multiplied by 1.03. The ratio of absorbance at 650 mμ (chlorophyll *b*) to 674 mμ (chlorophyll *a*) is 0.77 in heavy-water cells and 0.73 in ordinary-water cells. The absorbance in the 430-mμ region

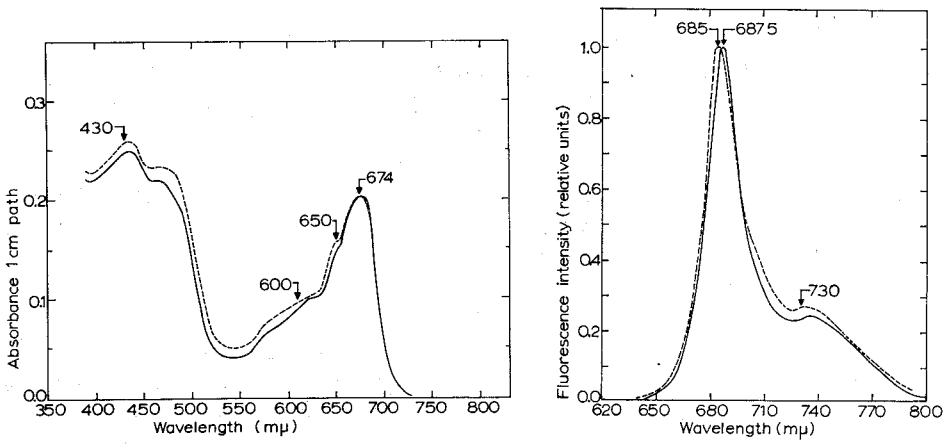

Fig. 1. Absorption spectra of deuterated (– – –) and hydrogen (———) *C. vulgaris*. The numbers on the graph show the location of certain points in mμ. The curves have been adjusted to give the same absorbance at 674 mμ.

Fig. 2. Emission spectra of deuterated (– – – –) and hydrogen (———) *C. vulgaris*. Wavelengths of exciting light: 600 mμ; temp. 22°. The curves have been "normalized" at 687 mμ.

is about 4% higher in deuterated cells, suggesting that it may contain a slightly higher proportion of carotenoids to chlorophyll *a*. The location of the absorption maxima seem to be the same in both cultures. However, a closer examination reveals that the deuterated cells have the blue chlorophyll *a* band at 434 mμ, whereas the hydrogen cells have the same band at 436 mμ. The absorbance curve of the deuterated cells lies under the curve of the hydrogen cells in the 674–700-mμ region, and lies above at wavelengths shorter than 674 mμ. This may be due to a slight shift of about 1 mμ of the chlorophyll *a* absorption band towards shorter wavelengths. However, minor shifts in the peak positions of the blue and the red bands may simply be due to variations in the relative concentrations of the different pigments.

Emission spectra

Upon excitation with 600-mμ light, the emission spectra of deuterated *C. vulgaris* show a peak at 686 mμ, whereas the emission spectra of hydrogen cultures have a peak at 687.5 mμ (Fig. 2). The measurements were made with the analyzing monochromator closed down to 2.0 mμ band widths, and the emission curves were normalized at 687 mμ. This shift of 1.5 mμ towards the shorter-wave side is correlated with a similar shift in the absorption spectrum. The ratio of fluorescence intensity at 730 mμ and the main fluorescence peak around 687 mμ was higher (0.30) in the deuterated sample, suggesting that it contains a larger proportion of the pigment responsible for fluorescence at 730 mμ; the ratio of fluorescence intensities at 687.5 and 730 mμ was 0.275 in the hydrogen sample.

The results obtained with 600-mμ excitation were confirmed upon excitation with 430 mμ (Fig. 3). The main emission peak was 687.5 mμ in hydrogen Chlorella cells, but it was shifted by 2.5 mμ towards the shorter wavelengths in the deuterated cells. The ratio of fluorescence intensities at 685 and 730 mμ was 0.27 in deuterated cells, whereas the ratio of fluorescence intensities at 687.5 and 733 mμ was 0.24 in hydrogen cells, again showing higher fluorescence yield in the long-wave region (with respect to the main emission peak) in the ^{2}H-containing cells.

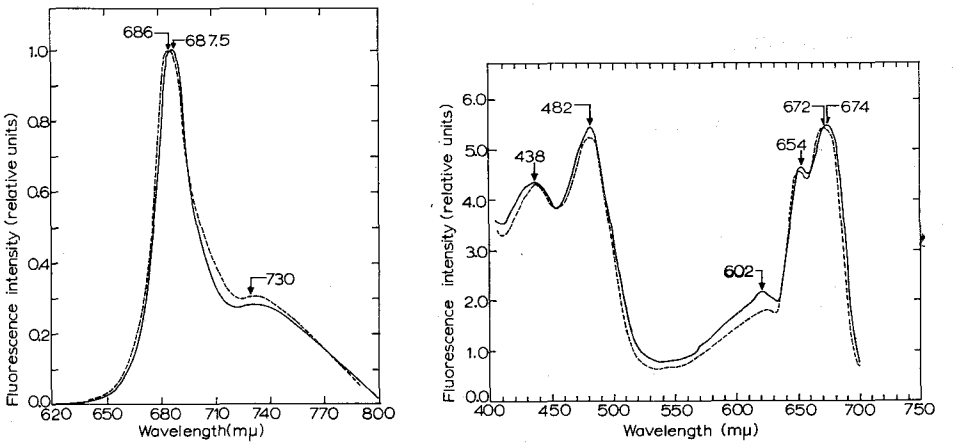

Fig. 3. Emission spectra of deuterated (– – – –) and hydrogen (———) *C. vulgaris*. Excitation at 430 mμ; temp. 22°. The curves have been normalized at 687 mμ.

Fig. 4. Excitation spectra (of fluorescence measured at 740 mμ) of deuterated (– – – –) and hydrogen (———) *C. vulgaris*.

In both cases, excitation with either 430- or 600-mμ radiation, the fluorescence yield was 20–30% greater in deuterated cells, as measured by the fluorescence intensity at the main emission peak.

The ratio of yields of fluorescence at 685 mμ upon excitation at 430 mμ and 600 mμ was calculated. First, corrections were made for the different incident quanta available at the two wavelengths and the different fractional absorption at 430 and 600 mμ. Without any further corrections, the ratio of the yields at 600 mμ to 430 mμ was 1.15 in deuterated samples and 1.43 in hydrogen samples. Since the quantum yield of photosynthesis measured by EMERSON AND LEWIS[7] as a function of wavelength suggests that carotenoids are only about 50% efficient in photosynthesis, the yield at 430 mμ must be somewhat lower, perhaps because of the presence of some inactive carotenoids.

Excitation spectra

Fig. 4 shows the action spectra (or the excitation spectra) of fluorescence measured at 740 mμ. The spectra for the H_2O-grown and ^2H_2O-grown cells were normalized at 672 mμ; the spectrum for hydrogen cells has been multiplied by 1.89. The yield in deuterated cells (measured at 740 mμ) was higher by a factor of 1.49 upon excitation at 670 mμ. A 2-mμ shift towards shorter wavelengths is noted in the peak due to chlorophyll *a* in the red end of the spectrum in the deuterated sample. This shift is correlated with the shift towards short wavelengths in the absorption spectrum and in the emission spectrum. A similar shift in the chlorophyll *b* band in the action spectrum of fluorescence was observed (Fig. 4). In the blue end of the action spectrum, no shift could be observed. Perhaps it is masked due to overlap with carotenoids.

A comparison of Fig. 4 with the absorption spectra of these samples (Fig. 1) suggests that the efficiency of energy transfer from chlorophyll *b* to chlorophyll *a* and from carotenoids to chlorophyll *b* must have been only slightly altered in deuterated samples.

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