THE APPROXIMATE RED ABSORPTION BAND OF CHLOROPHYLL *b* IN *ULVA LACTUCA* AT 77°K

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1. Introduction

The function of chlorophyll *b* in photosynthesis has been considered by various authors [1–6]. However, the absorption spectra of this pigment in vivo are not yet fully known. French [7] found by derivative spectrophotometry that in *Ulva*, additionally to the main one peaking at about 650 nm, a band shows up as a shoulder around 640 nm. Both bands have also been observed by Briantais [8] in corn chloroplasts, and by Litvin and Gulyaev [9, 10] in bean leaf preparations, but in *Chlorella* only the main maximum around 650 nm could be detected [10]. French and coworkers [11], and Michel and Michel-Wolwertz [12] observed both bands in an *Oenothera* mutant and spinach, respectively. They suggested that the occurrence of the 640 nm band is restricted to photosystem 2. Moreover, they pointed out that this additional band is not yet definitely proven to be due to a chlorophyll *b* component. The above-mentioned studies refer to normal absorption spectra of chloroplasts [8], derivative spectra [7, 9, 10] and difference spectra between both photosystems [11, 12].

Yentsch and Guillard [13], Thomas and Bielen [14], and Szász and Horváth [15] obtained "isolated" absorption spectra of chlorophyll *b* in *Stichococcus cylindrica*, spinach, and peas, respectively, as difference spectra between chlorophyll *b*-containing and chlorophyll *b*-free chloroplast preparations at room temperature. Thomas and Bretschneider [16], using the same technique, measured low-temperature absorption spectra of chlorophyll *b* in spinach. However, they did not compensate for an absorption band around 660 nm, probably due to chlorophyll *a*. Moreover, the 640 nm band did not show up sufficiently distinctly to be taken into consideration. Since this band is quite evident in *Ulva* [7], this seaweed was used in the present study to obtain the shape of the main chlorophyll *b* component at 77°K at close approximation.

2. Materials and methods

Ulva lactuca, obtained from the Netherlands Institute for Sea Research, Den Helder, was used either fresh or after storage at −40°C. Thalli, washed in 0.02 M phosphate buffer, pH 7.3, were macerated in a Sorvall Omnimixer. After filtration and centrifugation at about 50,000 *g* for 15 min, the chloroplast fragments were taken up in the mentioned buffer. For preparation of chloroplast fragments from the chlorophyll *b*-free algae *Vischeria stellata* and *Tribonema aequale* see ref. [16]. Absorption spectra at 77°K were recorded in a Cary Model 14R spectrophotometer, provided with a cooling attachment. In order to prevent cracks in the glassy preparation, high grade glycerol was added up to 65%, and 1 mm perspex cuvettes were used. The spectra of the chlorophyll *b*-free algae were made to coincide with those of *Ulva* around 668 nm, and subtracted as indicated in [16].

3. Results

An example of low-temperature absorption spectra of *Ulva lactuca* and *Vischeria stellata* is given in fig. 1. Their difference spectrum is shown in fig. 2. The band beyond 672 nm is due to lack of compensation in the

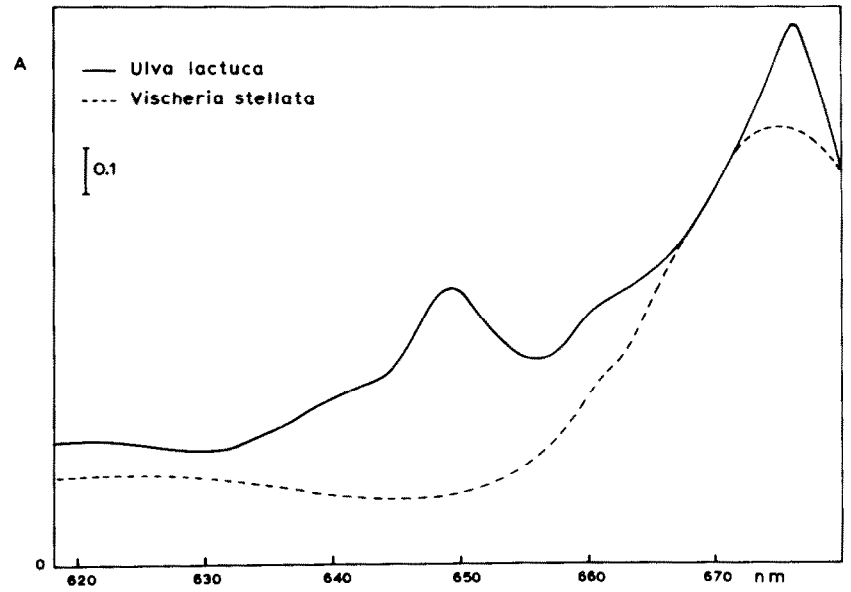

Fig. 1. Example of absorption spectra of *Ulva lactuca* and *Vischeria stellata* at 77°K.

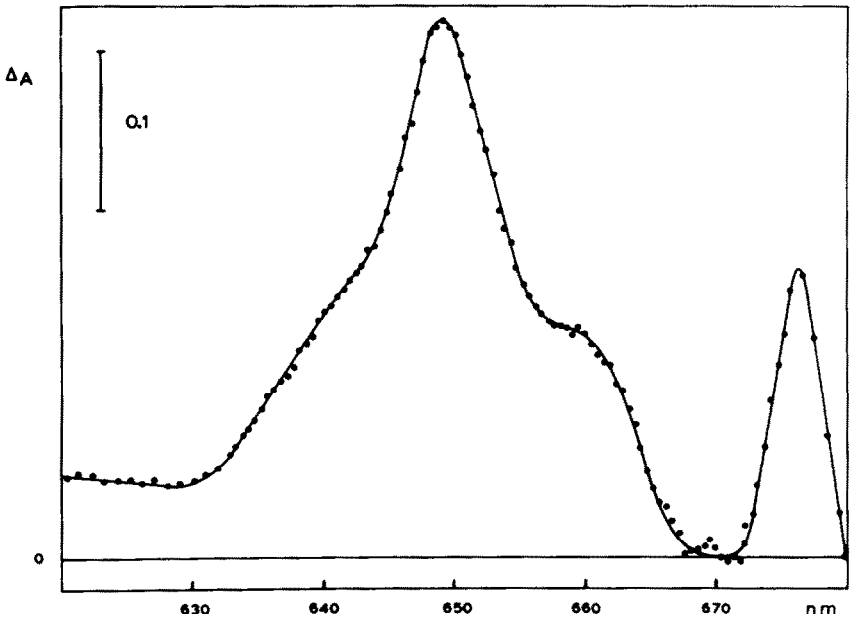

Fig. 2. Difference spectrum obtained from the spectra in fig. 1.

region of maximum chlorophyll *a* absorbance. The shoulder around 660 nm is due to a component different from chlorophyll *b*, probably the chlorophyll *a* type C_a665, peaking at 661 nm at 77°K, but not phytochrome [16]. The spectrum of this component was determined by subtracting spectra with unequal

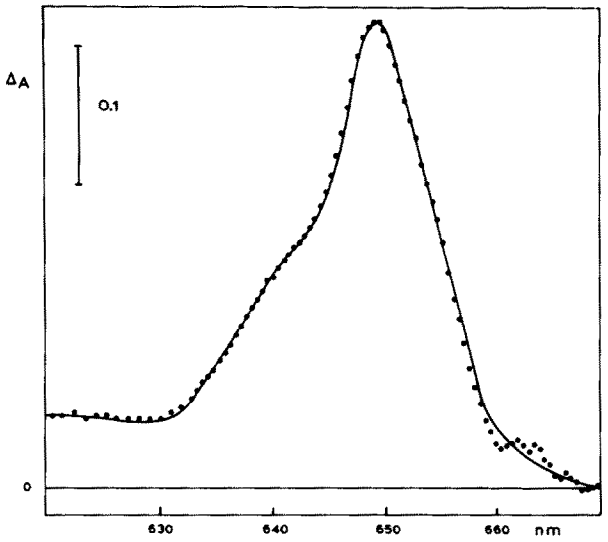

Fig. 3. The approximate chlorophyll *b* absorption spectrum in *Ulva lactuca* at 77°K, with the 640 nm component, and corrected for the 660 nm band. The chlorophyll *b* spectrum represents the mean of 10 spectra. The shape of the 660 nm band, used for subtraction, is averaged from 4 spectra.

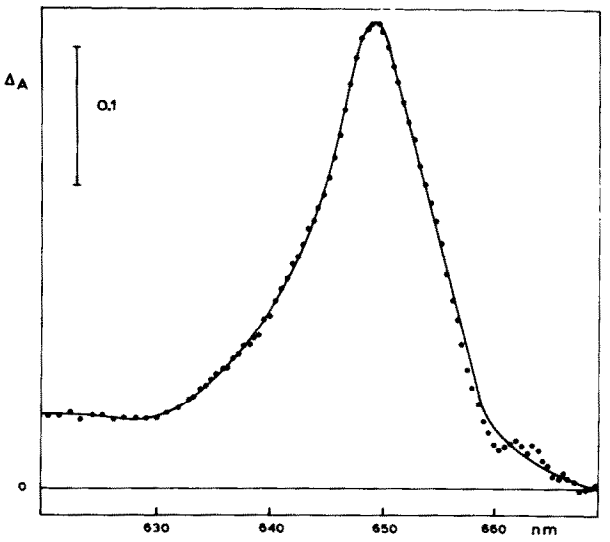

Fig. 4. The approximate shape of the main chlorophyll *b* absorption band in *Ulva lactuca* at 77°K. It is derived from the spectrum in fig. 3 by subtraction of the 640 nm band. The shape of the latter band is averaged from 4 spectra.

contributions. Subtraction of the thus-obtained spectrum from the chlorophyll *b* spectrum according to the best fit yielded the result given in fig. 3. The same was done for the 640 nm shoulder, shown in fig. 4. Preparations with different contributions of the 660 nm and 640 nm shoulders were obtained by either aerobic photobleaching or separation of photosystems 1 and 2 (to be published elsewhere).

4. Discussion

The difference spectra in figs. 3 and 4 clearly are approximations of the in vivo chlorophyll *b* absorption spectrum in *Ulva lactuca*. From fig. 2 it is evident that chlorophyll *a* absorbance cannot be completely compensated in the *Ulva* absorption spectrum. However, it has been concluded from experiments on aerobic photobleaching that the chlorophyll *a* compensation, made in the way described, is satisfactory [14, 16]. As yet, the 640 nm component cannot unambiguously be identified as a chlorophyll *b* form. All that can be said is that it is not likely to be due to a chlorophyll *a* type. For, if instead of *Vischeria, Tribonema* with a slightly different chlorophyll *a* absorption spectrum is used for compensation, a difference in the contribution of the 660 nm shoulder is observed, but the shapes of both the main maximum and the 640 nm shoulder remain the same [16]. Furthermore, the red absorption band of phytochrome, see ref. [16] and the related pigment allophycocyanin [17] are located at 660 nm and 654 nm respectively. Therefore, the component in question cannot be identical with these compounds either.

If, in fact, the 640 nm component refers to a chlorophyll *b* type, fig. 3 represents a close approximation of the overall chlorophyll *b* absorption spectrum in *Ulva lactuca* at 77°K. If the 640 nm component is restricted to photosystem 2 [11, 12], fig. 4 shows the closely approximate absorption spectrum of chlorophyll *b* from system 1 in this alga. The halfwidth value of this main red chlorophyll *b* absorption band is 13 nm.

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References

[1] M.B. Allen, L.R. Piette and J.C. Murchio, Biochim. Biophys. Acta 60 (1962) 539.
[2] B. Rumberg, Nature 204 (1964) 860.
[3] B. Rumberg and U. Siggel, Z. Naturforsch. 23b (1968) 239.
[4] H.T. Witt, Z. Naturforsch. 23b (1968) 244.
[5] H. Egneus, Physiol. Plantarum 21 (1968) 602.
[6] J. Keller and R. Bachofen, in: Progress in Photosynthesis Research, ed. H. Metzner, Vol. 2 (Tübingen, 1969) p. 1013.
[7] C.S. French, Brookhaven Symp. Biol. 11 (1958) 65.
[8] J.M. Briantais, Photochem. Photobiol. 6 (1967) 155.
[9] F.F. Litvin and B.A. Gulyaev, Nauchn. Dokl. Visshei Shkoly Biol. Nauki 2 (1969) 118.
[10] F.F. Litvin and B.A. Gulyaev, Dokl. Akad. Nauk. SSSR 189 (1969) 1335.
[11] C.S. French, J.S. Brown, L. Prager and M. Lawrence, Carnegie Inst. Year Book 67 (1969) 536.
[12] J.-M. Michel and M.-R. Michel-Wolwertz, Photosynthetica 4 (1970) 146.
[13] C.S. Yentsch and R.R. Guillard, Photochem. Photobiol. 9 (1969) 385.
[14] J.B. Thomas and J.W. Bielen, in: Progress in Photosynthesis Research, ed. H. Metzner, Vol. 2 (Tübingen, 1969) p. 646.
[15] K. Szász and I. Horváth, Acta Biochim. Biophys. Acad. Sci. Hung 5 (1970) 261.
[16] J.B. Thomas and F. Bretschneider, Biochim. Biophys. Acta 205 (1970) 390.
[17] C. O'hEocha, Arch. Biochem. Biophys. 73 (1958) 207.