*Acta Bot. Neerl.* 24(1), February 1975, p. 49–53.

# CURVE ANALYSIS OF THE RED ABSORPTION BAND OF CHLOROPHYLL *b* IN ULVA LACTUCA

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SUMMARY

A component absorbing around 655 nm is observed in the approximate red absorption band of chlorophyll *b* in *Ulva lactuca*. The results suggest that this component represents a form of chlorophyll *a* rather than chlorophyll *b*. Therefore, no indication that chlorophyll *b* occurs in more than a single form *in vivo* is obtained.

## 1. INTRODUCTION

In earlier papers (THOMAS & BRETSCHNEIDER 1970; THOMAS 1971a, b, 1974; LEPPINK & THOMAS 1973) the approximate shape of the *in vivo* red absorption band of chlorophyll *b*, obtained as a difference spectrum of the chlorophyll *b*-containing alga *Ulva lactuca* minus the chlorophyll *b*-free alga *Vischeria stellata* at 77 °K, was described. It was concluded that two shoulders around 641 nm and 662 nm respectively are not due to chlorophyll *b*, but, most likely, to chlorophyll *a* complexes. The present study aims at examining, by means of computer analyses, whether possibly hidden absorption bands occur in these chlorophyll *b* spectra.

## 2. MATERIALS AND METHODS

Chloroplast fragment suspensions from *Ulva lactuca*, obtained from the Netherlands Institute for Sea Research at Den Helder, are prepared according to previously described methods (THOMAS 1971a). However, instead of phosphate buffer, 0.05 M Tris buffer, pH 8.0 is used.

Fractionation into preparations enriched in either photosystem 1, the light fraction 1, or photosystem 2, the heavier fraction 2, is made according to BRIL et al. (1969) by using deoxycholate. The centrifugation procedure is slightly changed as described in THOMAS (1972). In addition fractionation is obtained by passing the chloroplast fragment suspensions through a French press twice, using a pressure of about 430 $kg.cm^{-2}$.

For the preparation of suspensions of chloroplast fragments from the chloro-

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phyll *b*-free alga *Vischeria stellata* see THOMAS & BRETSCHNEIDER (1970).

Absorption spectra at 77 °K are recorded in a Cary model 14R spectrophotometer. By subtracting absorption spectra of *Vischeria* from those of *Ulva*, as described in THOMAS & BRETSCHNEIDER (1970), spectra of chlorophyll *b in vivo* are obtained in close approximation.

The computer analyses are done with the RESOLV program developed by Dr. D. D. Tunnicliff of the Shell Development Laboratory, Houston, Texas. The version used is a revision of the original program and was kindly provided by Dr. C. S. French of the Carnegie Institution of Washington at Stanford, California, where the revisions were programmed. After some minor changes, due to the type of computer, it runs satisfactorily on a CYBER-73.

The scope of the program has been described in FRENCH et al. (1969, 1971, 1972). The spectra are assumed to fit a sum of simple components. In this case a mixture of a Gaussian and Lorentzian curve is chosen as the simple component. The curvefitting is an iteration process. With every step the difference between the calculated and the measured spectrum decreases by changing the parameters. The iteration is stopped when the improvement of the fit becomes less than 0.1 % or the number of steps exceeds the allowed maximum of 30. At the beginning the components are assumed to be 50 % Gaussian and 50 % Lorentzian. During the same iterationstep the parameters are not allowed to change more than 0.3 nm to 2 nm depending on the parameter.

The graphical output of the program is shown in *fig. 1*. The dots represent the measured spectrum, the drawn line is the calculated one. The simple components are given as well. The difference between both spectra is shown underneath. The scale of the lower plot is different from the scale in the upper plot by the indicated factor.

## 3. RESULTS

An example of curve analysis of the red absorption band of chlorophyll *b* in *Ulva lactuca* is given in *fig. 1*. In *fig. 1a*, resolution into 4 components is shown. The major one is the main chlorophyll *b* band, whereas both minor ones represent the 641 and 662 nm components. These are not due to chlorophyll *b*, but, most probably, to chlorophyll *a* complexes (THOMAS & BRETSCHNEIDER 1970; THOMAS 1971a, b; LEPPINK & THOMAS 1973). The band around 630 nm, only partly shown, is the result of the fact that the absorbance at the short-wave side of the spectrum does not approach zero. It therefore is not considered any further. At the short-wave side, the sum of the resolved components, shown by the drawn line, coincides satisfactorily with the measured values, represented by dots. This coincidence is not increased by a splitting of the 641 nm band, cf. *fig. 1b*. At the long-wave side, the coincidence is imperfect. To obtain a good fit, the introduction of a component peaking at 655 nm is required, cf. *fig. 1c*. At the same time, the fit at the short-wave side is also slightly improved. Since splitting of the 641 nm band does not ameliorate the mentioned coincidence, *fig. 1d* shows the final analysis. The 655 nm band occurs in all, 20, analyzed spectra.

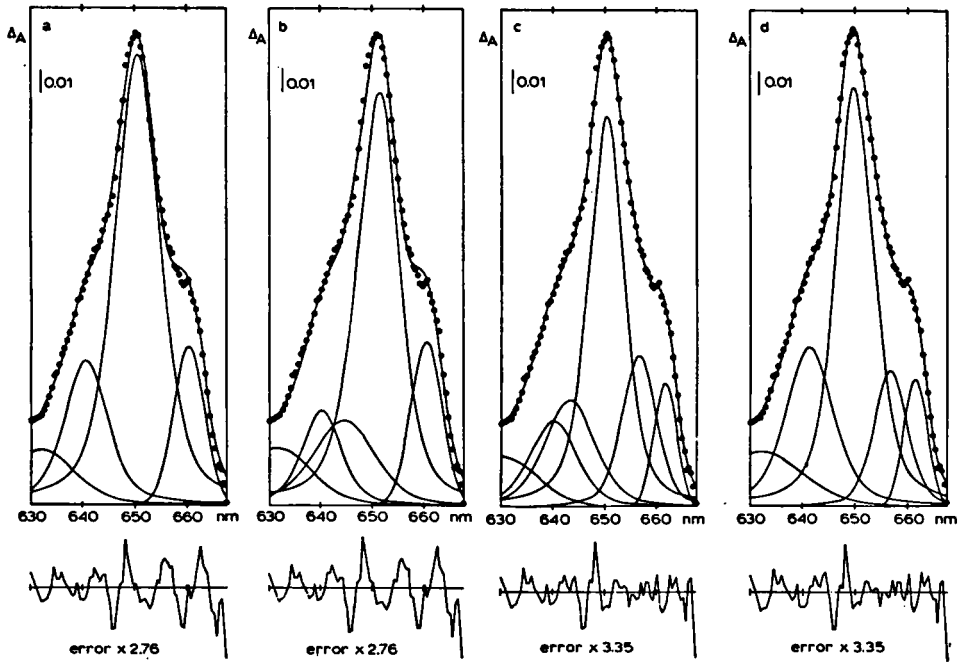


Fig. 1. Example of curve resolution of chlorophyll *b* absorption spectra from *Ulva lactuca*. For details see text.

In order to examine a possible association of the 655 nm component with one of both photosystems, fractionation experiments are done. As observed earlier (THOMAS 1972), separation of both photosystems, even with deoxycholate, is only poor for *Ulva*. The results, shown in *table 1*, suggest that the 655 nm component occurs to a higher proportion in fraction 1, enriched in photosystem 1, than in fraction 2. Upon equalizing the heights of the main red chlorophyll *a* absorption bands around 677 nm for all preparations, the relative heights of the 655 nm bands are given in mm.

Table 1. Relative amount of the 655 nm component in fractions enriched in one of both photosystems. Data refer to 77°K. DOC; deoxycholate, F.p.: French press.

| fractionation procedure | height of the 655 nm absorption band in relative units mm | |
|---|---|---|
| | fraction 1 | fraction 2 |
| DOC | 27.8 | 23.2 |
| DOC | 33.4 | 19.4 |
| DOC | 24.5 | 22.6 |
| F.p | 21.6 | 20.0 |
| F.p | 24.8 | 23.2 |
| mean | $26.4 \pm 2.0$ | $21.7 \pm 0.8$ |

## 4. DISCUSSION

Resolution into mixed Gaussian and Lorentzian bands of spectra as shown in *fig. 1* demonstrates that, in addition to the main chlorophyll *b* band, peaking at 649 nm, as well as the bands due to chlorophyll *a* forms (THOMAS & BRETSCHNEIDER 1970; THOMAS 1971a, b; LEPPINK & THOMAS 1973) with maxima at 641 and 662 nm respectively, a component with maximum absorption at 655 nm occurs in *Ulva lactuca*. FRENCH et al. (1969, 1971, 1972) and WIESSNER & FRENCH (1970) also observed a 655 nm component by the curve resolution method in various species. However, adequate fits could be realized in a number of cases without the addition of the 655 nm component. For this reason, the 655 nm band was not considered by these authors any further. The present results, however, show that this component occurs in *Ulva* without any doubt.

J. THOMAS et al. (1970, a b) and TATAKE et al. (1971) made microspectrophotometric studies on algal cells at room temperature. These authors concluded from derivative spectrophotometric results to the occurrence of a 655 nm band. They suggested that this component represents a chlorophyll *b* form. However, the fact that, in contrast with chlorophyll *b*, the 655 nm component is likely to occur more abundantly in photosystem 1 than in photosystem 2 renders it less probable that this component represents a chlorophyll *b* form. Such a situation would mean that chlorophyll *b* occurs only in a single form *in vivo*. In this respect it is mentioned that KLEUSER & BÜCHER (1969), when studying electrochromic effects in monomolecular films of chlorophylls *a* and *b*, observed that, contrary to the first pigment, the latter one occurred in a single aggregation form. About the nature of the 655 nm component it is mentioned that, according to DÖRING et al. (1969) the first vibrational band of P700 occurs at 655 nm. This fact, combined with the present indication that the 655 nm component occurs preferably in fractions enriched in photosystem 1 points to the possibility that the component in question is due to the reaction center pigment of this photosystem, its collector pigment C700 (KOK 1966), or to both of these.

ACKNOWLEDGEMENT

Thanks are due to Miss. W. Baas for skillful technical assistance.

REFERENCES

BRIL, C., D. J. VAN DER HORST, S. R. POORT & J. B. THOMAS (1969): Fractionation of spinach chloroplasts with sodium deoxycholate. *Biochim. Biophys. Acta* **172**: 345–348.

DÖRING, G., G. RENGER, J. VATER & H. T. WITT (1969): Properties of the photoactive chlorophyll-aII in photosynthesis. *Z. Naturforschg.* **24b**: 1139–1143.

FRENCH, C. S., J. S. BROWN, L. PRAGER & M. C. LAWRENCE (1969): Analysis of spectra of natural chlorophyll complexes. *Carnegie Inst. Wash. Year Book* **67**: 536–546.

—, —, W. WIESSNER & M. C. LAWRENCE (1971): Four common forms of chlorophyll a. *Carnegie Inst. Wash. Year Book* **69**: 662–670.

—, — & M. C. LAWRENCE (1972): Four universal forms of chlorophyll *a*. *Plant Physiol.* **49**: 421–429.

KLEUSER, D. & H. BÜCHER (1969): Elektrochromie von Chlorophyll-a und Chlorophyll-b in monomolekularen Filmen. *Z. Naturforschg.* **24b**: 1371–1374.

KOK, B. (1966): Concentration and normal potential of primary photo-oxidants and reductants in photosynthesis. In: *Currents in photosynthesis* (J. B. THOMAS and J. C. GOEDHEER, eds.). Ad. Donker Publisher, Rotterdam. pp. 383–391.

LEPPINK, G. J. & J. B. THOMAS (1973): On the identity of the 640-nm component observed in chlorophyll *b* absorption spectra in vivo. *Biochim. Biophys. Acta* **305**: 610–617.

TATAKE, V. G., J. THOMAS, G. P. PHONDKE & A. R. GOPAL-AYENGAR (1971): Derivative microspectrophotometry of single cells in vivo. *Exptl. Cell Res.* **66**: 251–253.

THOMAS, J., G. P. PHONDKE, V. G. TATAKE & A. R. GOPAL-AYENGAR (1970a): Microspectrophotometric studies on the pigments in vivo of single algal cells. I. Pigments of Chlorella pyrenoidosa. *Photochem. Photobiol.* **11**: 85–92.

—, —, — & — (1970b): Microspectrophotometric studies on the pigments in vivo of single algal cells. Part II-Multiple forms of chlorophyll in Scenedesmus obliquus & Chlamydomonas sp. *Indian J. Exptl. Biol.* **8**: 211–214.

THOMAS, J. B. (1971a): The approximate red absorption band of chlorophyll *b* in Ulva lactuca at 77°K. *FEBS Letters* **14**: 61–64.

— (1971b): On the in vivo absorption spectrum of chlorophyll *b*. In: *Proc. 1st Eur. Biophys. Congr. Baden near Vienna, Austria,* (E. BRODA, A. LOCKER and H. SPRINGER-LEDERER, eds.) Wiener Medizinische Akademie, Wien. Vol. **4**: 37–41.

— (1972): Chlorophyll *b* in preparations of photosystems I and II. In: Proc. 2nd Int. Congr. Photosynthesis Research, Stresa, 1971. (G. Forti, M. Avron and A. Melandri, eds.) Dr. W. Junk N. V. Publishers, The Hague. pp. 1509–1513.

— (1974): Further data on the identity of a pigment absorbing around 640 nm in Ulva lactuca. *Biochim. Biophys. Acta* **333**: 415–420.

— & F. BRETSCHNEIDER (1970): A study of chlorophyll *b* in vivo. *Biochim. Biophys. Acta* **205**: 390–400.

WIESSNER, W. & C. S. FRENCH (1970): The forms of native chlorophyll in Chlamydobotris stellata and their changes during adaptation from photo-heterotrophic to autotrophic growth. *Planta* **94**: 78–90.