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NEW CHLOROPHYLL-*b* FORMS IN A CHLOROPHYLL-DETERGENT PHOTOSYNTHETIC MODEL SYSTEM

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SUMMARY

The absorption spectra of chlorophyll *b* in Triton X-100 micelles at room temperature are superpositions of components with absorption maxima at 640.8, 648.9, 659.5, 669.6, 682.1 and 695.7 nm, obtained from Gaussian analysis of the spectra. The last four forms strongly overlap the chlorophyll *a* forms of this system obtained with maxima at 659.3, 667.6, 674.3, 680.8, 686.5, 692.8, 701.9, 713.6 and 722.0 nm.

Since the in vivo chlorophyll *a* forms practically coincide with the forms found in this system, the possible existence of in vivo overlapping chlorophyll *b* and *a* forms eventually should be taken into consideration. In this case, however, the Gaussian analysis of in vivo absorption bands in itself in the proper spectrum range cannot discriminate between chlorophyll *a* and *b* components.

INTRODUCTION

The red absorption band of photosynthetizing organisms has been extensively studied. These studies lead to the conclusion that the red absorption band is composed of several in vivo chlorophyll forms characterized by different peak wavelengths and half-band widths. According to French et al. [1] four universal in vivo chlorophyll *a* forms exist with absorption maxima at 661.6, 669.0, 677.1, 683.7 nm. In addition, two forms at 691.5 and 704 nm are found in several organisms. Gulyaev and Litvin [2] found the forms 662, 670, 676, 683, 686, 693, 702, 712 and 720 nm. Also two chlorophyll *b* forms at 640 and 650 nm were reported by Butler and Hopkins [3] and French et al. [1]. Thomas and Bretschneider [4], and Leppink and Thomas [5] believe, however, in the existence of a single 650 nm chlorophyll *b* form. Oudshorn and Thomas [6] suggested the existence of a single chlorophyll *b* form at 649 nm in *Ulva lactuca* based upon curve analysis of the red absorption band.

In nonpolar solvents, Cotton et al. [7] obtained five Gaussian components of chlorophyll *a* around 626, 650, 667, 682 and 695 nm. Using these components, the in vivo red band of algae could be deconvoluted. Iriyama [8] found several chlorophyll *b* forms (643, 650, 672 and 680 nm) in solutions from a qualitative analysis of the

changes in the absorption spectrum. Kleuser and Bücher [9], when studying electrochromic effects in monomolecular films of chlorophyll *a* and *b*, observed that, contrary to the first pigment, the latter one occurred in a single aggregation form.

Though the structural difference between chlorophyll *a* and *b* is very small, their physical, chemical and biological properties seem to be rather different. Chlorophyll *b* has seldom been studied in comparison with chlorophyll *a* in model systems. We performed absorption measurements on both in detergent micellar solutions.

METHODS

The technique of preparation of chlorophylls, the micellar solutions and the method of measurements are described elsewhere [10, 11]. Alysentsev's method [12] was employed in order to resolve the bands. According to this method, the number and location of the components can be determined without any preliminary arbitrary assumption. The method is based on the possibility of presenting the individual spectra as linear combinations of separate bands and graphically solving the set of linear equations obtained. The number and location of the components is independent of the temperature. Starting from the data obtained by this method, computer analysis was carried out on the basis of the damped least squares method given by Marquardt [13]. Following consecutive runs of numerous bands of different chlorophyll *a*, *b* and detergent concentrations, the analysis was performed by means of a CDC 3300 computer. The experimental spectra were taken at room temperature.

RESULTS AND DISCUSSION

Concentration-dependent changes in the red absorption bands of the chlorophyll-detergent model studied by us [10, 14, 15] indicated the presence of chlorophyll forms and their transformation from one into another. These bands are shown in Fig. 1 in the present system.

The parameters obtained for computer analysis from several runs are entered in Table I. From Table I it may be seen that the computer deconvolution produced

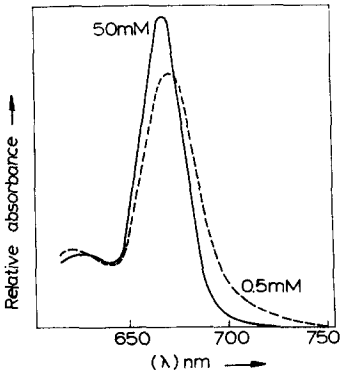

Fig. 1. Relative absorption spectra of 0,2 mM chlorophyll *a* with 0.5 and 50 mM Triton X-100 in water.

TABLE I

LOCATIONS OF MAXIMA AND BAND WIDTHS OF GAUSSIAN COMPONENTS OF THE RED ABSORPTION BANDS OF CHLOROPHYLL *a* AND CHLOROPHYLL *b*

Chlorophyll *a*		Chlorophyll *b*	
Wavelength of maximum (nm)	Halfwidth (nm)	Wavelength of maximum (nm)	Halfwidth (nm)
–	–	605,6	34,8
–	–	628,4	27,2
633,0	20,4	640,8	17,0
648,3	18,0	648,9	16,2
659,3	14,8	659,5	17,7
667,6	13,9	669,6	18,8
674,3	11,7	682,1	23,9
680,8	10,0	695,7	35,5
686,5	9,2	–	–
692,8	10,4	–	–
701,9	12,5	–	–
713,6	16,6	–	–
722,0	14,6	–	–

practically the same chlorophyll *a* forms as are found by Gulyaev and Litvin [2]; the six universal forms of French et al. [1] are also well approximated. Fig. 2. shows the distribution and the relative heights of the components. From the agreement between the in vivo findings and our present data, it seems that the pigment-detergent model is capable of producing the pigment forms supposed to be functioning in the in vivo systems. This is not surprising, since it has been shown earlier that within the micelles, locally, there appear in vivo pigment concentrations [10, 11].

If chlorophyll *b* is incorporated into the detergent micelles, the red band of absorption behaves similarly to the red band of chlorophyll *a* (Fig. 3). As has already

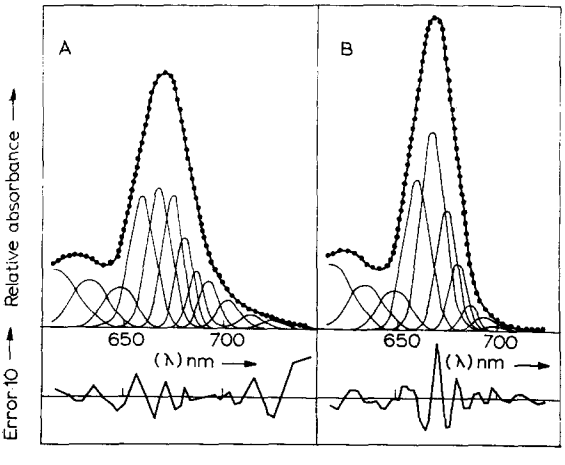

Fig. 2. Gaussian components of the relative absorption spectra of 0,2 mM chlorophyll *a*: A, with 0,5 mM; B, with 50 mM Triton X-100 in water.

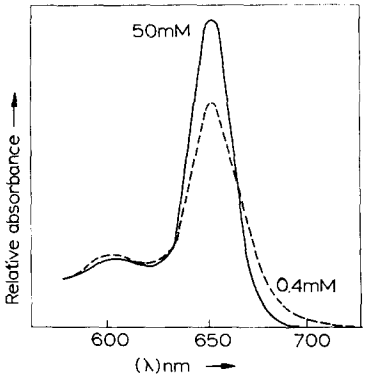

Fig. 3. Relative absorption spectra of 0,1 mM chlorophyll *b* with 0,4 and 50 mM Triton X-100.

been published [10], the band width and the location of the bands change with the concentration of chlorophyll *b*. This points to the fact that chlorophyll *b* should have several spectral forms in addition to those already known. [1, 2, 4, 5].

The former process of spectrum analysis carried out for chlorophyll *b* led to the results shown in Fig. 4. It is seen that in this in vitro system four to six additional forms, never observed before under in vivo conditions, are obtained overlapping with the chlorophyll *a* forms. In the in vivo systems where both chlorophyll *a* and chlorophyll *b* are present, the incorporation of chlorophyll *b* into the chlorophyll *a* antenna the spectral behavior of chlorophyll *a* will not be radically affected [6, 16]. According to our measurements, this seems to be true also for mixtures of chlorophyll *a* and *b* in detergent micelles from the additivity of the absorption spectra. Consequently, in our

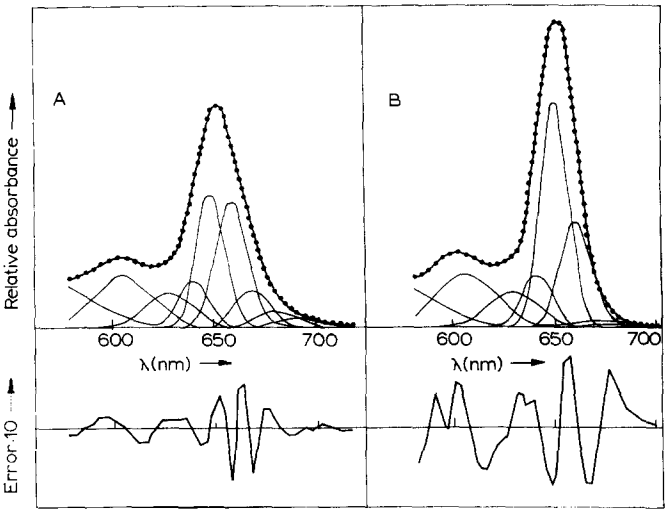

Fig. 4. Gaussian components of the relative absorption spectra of 0,1 mM chlorophyll *b*: A, with 0.4; B, with 50 mM Triton X-100.

in vitro system the different chlorophyll *a* and *b* forms should be present almost independently. This, in turn, renders the analysis of the red band of these systems practically impossible in the spectrum range where both pigments possess component forms. The question arises whether in the in vivo systems containing chlorophyll *a* and *b*, the Gaussian analysis of the red band is able in itself discriminate among chlorophyll *a* and *b* spectral forms. We believe that the discrimination can be satisfactorily achieved by changing appropriate biological parameters.

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REFERENCES

1 French, C. S., Brown, J. and Lawrence, M. (1972) Plant Physiol. 49, 421–429
2 Gulyaev, B. A. and Litvin, F. F. (1970) Biofizika 15, 670–680
3 Butler, W. L. and Hopkins, D. W. (1970) Photochem. Photobiol. 12, 451–456
4 Thomas, J. B. and Bretschneider, F. (1970) Biochim. Biophys. Acta 205, 390–400
5 Leppink, G. J. and Thomas, J. B. (1973) Biochim. Biophys. Acta 305, 610–617
6 Oudshoorn, H. P. and Thomas, J. B. (1975) Acta Bot. Neerl. 24, 49–53
7 Cotton, T. M., Trifunac, A. D., Ballschmitter, K. and Katz, J. J. (1974) Biochim. Biophys. Acta 368, 181–198
8 Iriyama, K., Yoshiura, M. and Asai, M. (1974) Chem. Lett. (Japan) 997–1000
9 Kleuser, D. and Bücher, H. (1969) Z. Naturforsch. 24b, 1371–1374
10 Lehoczki, E. and Csatorday K. (1975) Biochim. Biophys. Acta 396, 86–92
11 Csatorday, K., Lehoczki, E. and Szalay, L. (1975) Biochim. Biophys. Acta 376, 268–273
12 Fok, M. B. (1972) Trudy Institute Fyziki Akad. Sci. U.S.S.R. 59, 3–24
13 Marquardt, D. W. (1963) J. Soc. Ind. Appl. Math. 11, 431–441
14 Lehoczki, E., Hevesi, J. and Tolvaj, L. (1974) Acta Phys. Pol. A46, 741–748
15 Szabad, J., Lehoczki, E., Szalay, L. and Csatorday, K. (1974) Biophys. Struct. Mech. 1, 65–74
16 Dinant, M. and Aghion, J. (1975) Eur. J. Biochem. 52, 515–520