Acta Bot. Neerl. 26(4), August 1977, p.299–302.

INTRAMEMBRANOUS PARTICLES AND CHLOROPHYLL COMPLEXES IN CHLAMYDOMONAS SPEC.*

W. VERWER** and J W. KLEINEN HAMMANS

Vakgroep Biofysica, Laboratorium voor Experimentele Fysica, Utrecht

SUMMARY

The marine alga *Chlamydomonas* spec. contains a very high concentration of chlorophyll *b*. Electron micrographs of freeze-fracture preparations show that membrane particles occur in two sizes and in two different population densities. Computer analysis of a low temperature absorption spectrum shows a high amount of chlorophyll *b* but only moderate amounts of chlorophyll *a*-640 and of chlorophyll *a*-685.

1. INTRODUCTION

In an earlier study (see VERWER et al. 1977) we investigated a possible relationship between chloroplast structure and pigment composition. To this end low temperature absorption spectra were recorded and particle population densities and sizes were measured in electron micrographs of freeze-fractured preparations obtained from four plant species. One of these (*Tribonema aequale*) holds a special position, since it contains no chlorophyll *a*-640 (C_a640). no C_a685 and no chlorophyll *b* (C_b). Since *Chlamydomonas* spec. contains a relatively large amount of C_b we thought it interesting to compare the pigment composition and the sizes and population densities of its membrane particles with the same aspects of the species studied previously.

2. MATERIAL AND METHODS

Chlamydomonas spec. was isolated from water from the Waddenzee near Den Helder, and grown on a solid agar medium of artificial seawater.

For electron microscopy cells were removed from the agar by shaking the culture tube containing the agar with a solution of 3% NaCl and 25% glycerol in water. The resulting cell suspension was centrifuged for 5 min at 12000 × g in a Sorvall RC-2B centrifuge. The cell sediment was frozen in a slurry of solid and liquid nitrogen at −210°C. The frozen samples were fractured using a

* Dedicated to Professor Dr. J. B. Thomas, upon his retirement from the chair of Biophysics.

** Postal address: Physics Laboratory, State University, Sorbonnelaan 4, Utrecht, The Netherlands.

Denton apparatus at −100°C. Next platinum and carbon were evaporated in order to obtain a replica of the fracture surface. The platinum replicas were cleaned in concentrated sulphuric acid, and then treated with a hypochlorite solution. The replicas were then transferred to copper grids and studied in a Philips EM 301 electron microscope. Particle diameters in the micrographs were determined with a binocular microscope BM-51-2 (magnification about 9×) equipped with a micrometer grating. The magnification in the micrograph was about 60000×. According to ELBERS & PIETERS (1964) this number is inherently inaccurate to about 10%.

Absorption spectra of whole cells suspended in 60% glycerol were recorded at low temperature (−196°C) in a Shimadzu MPS-50L spectrophotometer. The spectra were analyzed in a Cyber-73 computer using the RESOLV program developed by Dr. D. D. Tunnicliff of the Shell Development Laboratory, Houston, Texas and revised by Dr. C. S. French and Mr. H. P. Oudshoorn. For the scope of the program see FRENCH et al. (1969), FRENCH et al. (1972) and OUDSHOORN & THOMAS (1975). The spectral region studied, ranged from 600 nm to 750 nm.

3. RESULTS

3.1. Spectra

The low temperature absorption spectrum of *C.* spec. together with the computer analysis is given in *fig. 1. Table 1* summarizes some additional results.

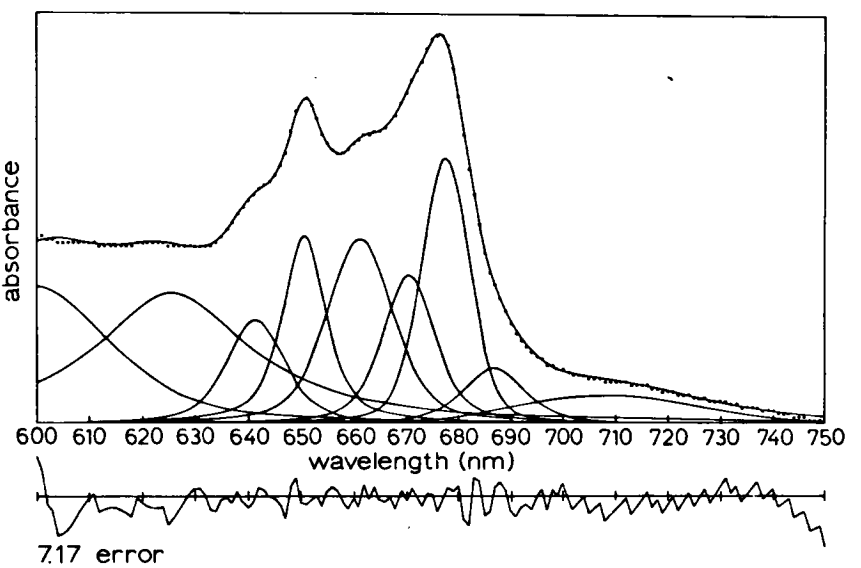

Fig. 1. Absorption spectrum of *C.* spec. at −196°C with computer analysis. Shown below is the difference between the actual spectrum and the sum of the calculated components, multiplied by 7.17.

Table 1. Computer analysis of the low-temperature absorption spectrum of *C.* spec.

Band name	Band centre nm	Halfwidth nm	Area %
C_b	650.6	9.6	17.4
C_a640	641.5	12.7	11.9
C_a662	661.3	14.9	23.9
C_a670	670.5	11.7	15.7
C_a680	677.6	10.7	23.5
C_a685	686.7	13.8	7.1

Attention should be given to the fact that the amount of C_b is high and that the amount of C_a685 is low.

3.2 Electron microscopy

The membrane particles as seen in our micrographs (see e.g. *fig. 2*) can be divided into two groups. Those in the first group (EF face, BRANTON et al. (1975)) have a mean diameter of 13.0 ± 0.3 nm. They are either densely packed, 40 particles per 10^{-2} μm², in the stacked region (GOODENOUGH & STAEHELIN 1971) or widely dispersed, 1 to 8 particles per 10^{-2} μm², in the unstacked region. The particles in the other group (PF face) have a mean diameter of 8.5 ± 0.5 nm. They are generally densely packed, 60 particles per 10^{-2} μm². The particle diameter ratio of 0.65 ± 0.4 and the density ratio is 1.5 ± 0.2.

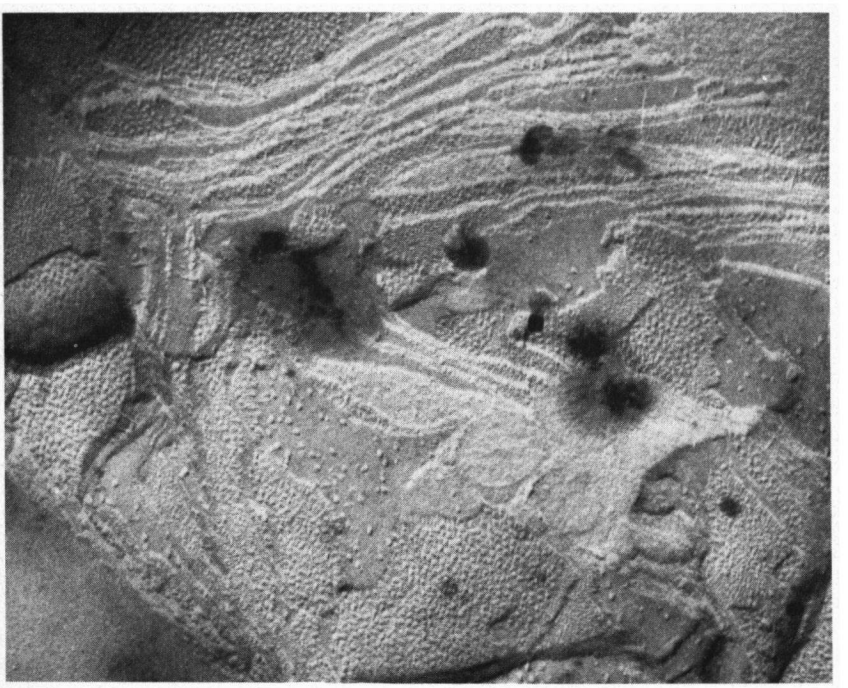

Fig. 2. Freeze-fracture thylakoids of *C. spec.* Magnification, + 60000.

4. DISCUSSION

KLEINEN HAMMANS & THOMAS (1976) investigated the amounts of C_b and C_a forms in nine plant species. They found that in these species the amount of C_b, as expressed in percentage of the area of the red absorption band, ranges from 0.00 to 15.75%. This result places *C.* spec. with 17.4% C_b in an extreme position. The amount of C_c640 is not very high in *C.* spec. The amount of C_a685 is low, so we may conclude that the suggestion of KLEINEN HAMMANS & THOMAS (1976) concerning a complex formed by C_b, C_a640 and C_a685 is not supported by the analysis of the low-temperature absorption spectrum of *C.* spec. We do not exclude the possibility that in *C.* spec. only part of the C_b occurs in a complex with C_a685 and C_a640. As mentioned in the introduction (see VERWER et al. 1977) we investigated a possible relationship between pigment composition and ultrastructure of chloroplasts of four of the nine plant species just mentioned. In that study we found a tendency for plants with larger amounts of C_b to show smaller particle diameter ratios and particle density ratios. This tendency is confirmed by the data obtained from *C.* spec.

ACKNOWLEDGEMENTS

We are grateful to Ms. J. Leunissen for her skilful technical assistance, to Dr. D. D. Tunnicliff and Dr. C. S. French for providing the RESOLV computer program, to Dr. P. H. Nienhuis for determining the genus of the alga used and to Ms. S. M. McNab, M. A. for making helpful and constructive comments on the language and style of this article.

REFERENCES

BRANTON, D., S. BULLIVANT, N. B. GILULA, M. J. KARNOVSKY, H. MOOR, K. MÜHLETHALER, D. H. NORTHCOTE, L. PACKER, B. SATIR, P. SATIR, V. SPETH, L. A. STAEHELIN, R. L. STEERE & R. S. WEINSTEIN (1975): Freeze-etching nomenclature. *Science* **190**: 54–56.

ELBERS, P. F. & J. PIETERS (1964): Accurate determination of magnification in the electron microscope. *J. Ultrastruc. Res.* **11**: 25–32.

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

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

GOODENOUGH, U. W. & L. A. STAEHELIN (1971): Structural differentiation of stacked and unstacked chloroplast membranes, Freeze-etch electron microscopy of wild-type and mutant strains of Chlamydomonas. *J. Cell Biol.* **48**: 594–619.

KLEINEN HAMMANS, J. W. & J. B. THOMAS (1976): On the correlation between the amounts of chlorophyll *b* and chlorophyll *a* forms in various plants. *Acta Bot. Neerl.* **25** (1): 63–69.

OUDSHOORN, H. P. & J. B. THOMAS (1975): Curve analysis of the red absorption band of chlorophyll *b* in Ulva lactuca. *Acta Bot. Neerl.* **24** (1): 49–53.

VERWER, W., J. W. KLEINEN HAMMANS, J. B. THOMAS & P. H. J. TH. VERVERGAERT (1977) *Biochim. Biophys. Acta* (in press).