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INTRAMEMBRANOUS PARTICLES AND CHLOROPHYLL COMPLEXES IN CHLOROPLASTS

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SUMMARY

The size and population density of large and small particles from freeze-fractured chloroplasts of three wild-type algae and of normal spinach were determined.

Computer analyses of low-temperature absorption spectra of chloroplast preparations from these species were performed, and a possible correlation between the occurrence of seven chlorophyll complexes and the aforementioned properties of the intramembranous particles was studied.

It was found that only single-sized particles occur in a species containing neither chlorophyll *b* nor chlorophyll *a*-685 complexes. The three remaining species carry particles of two sizes, termed large and small particles. However, from quantitative considerations it is concluded that the chlorophyll content of none of the various pigment complexes is related to the size and the population density of the studied particles. If such a relationship exists, it seems likely to be due to the carrier moiety of the chlorophyll *b* chlorophyll *a*-685 complex.

INTRODUCTION

Various authors (cf. ref. 1) have studied a possible relationship between the occurrence of chlorophyll complexes and chloroplast substructure. To this end, properties of chloroplasts from mutants were compared with those from the corresponding wild-type species Many results are, however, contradictory. For instance, according to a number of authors [2–5] a relationship may exist between the occurrence of chlorophyll *b* and the chloroplast substructure. However, according to others [6–8] no such relationship exists Boardman et al. [4, 5] observed the absence of large intramembranous particles in chloroplasts from a chlorophyll *b*-free barley mutant, studied by Highkin [9], whereas Henriques and Park [10], using the same mutant, did not observe any change in either density or size of the particles as compared to the wild type. Thornber and Highkin [11] found that absence of chlorophyll *b*

in the mentioned mutant is accompanied by loss of chlorophyll *a* and carotenoids as well as the protein moiety of the light-harvesting pigment complex, previously termed PSII complex.

Boardman et al. [4, 5] and Remy and Bebee [12], studying irradiation effects on wild-type species, reported variations of the chlorophyll *a*/chlorophyll *b* ratio induced by changing the light conditions. Therefore, it may be worthwhile to examine a possible relationship between chloroplast substructure and occurrence of the various chlorophyll *a* complexes.

The present study investigates any possible relationship between size and population density of the chloroplastic intramembranous particles, visualized in freeze-fracture preparations, and the amount of the chlorophyll *b* · protein complex and of the various chlorophyll *a* forms. In the investigations cited, properties from mutants were compared with those of the corresponding wild-type species. As mentioned already [10] there is a definite possibility that some lesions, other than chlorophyll *b* deficiency, exist on the photosynthetic apparatus. The conflicting conclusions mentioned above might be ascribed to such a possibility Therefore only wild-type species, three algae and one higher plant, containing different amounts of chlorophyll *b*, are used in the present experiments.

MATERIALS AND METHODS

Three algae, *Ulva lactuca*, *Euglena gracilis*, and *Tribonema aequale*, as well as the higher plant *Spinacia oleracea* (L var. Noorman) were used. The four species were grown at the institute.

Chloroplast fragment suspensions were obtained from *Tribonema* by sonication (cf. ref. 13), from *Euglena* by grinding in a mortar, (cf ref. 14), and from *Ulva* as well as spinach by macerating in a Sorvall Omnimixer, (cf. ref. 15). Except for *Tribonema* these preparations were used for both electron microscopical study and establishing of absorption spectra. For electron microscopy whole *Tribonema* cells were used.

All chloroplast fragment samples were prepared and finally suspended in 0 02 M phosphate buffer, pH 7.3. For low-temperature absorption spectra reagent-grade glycerol was added to a final concentration of 66 %.

Electron microscopy

Samples were frozen in a slurry of solid and liquid nitrogen at −210 °C. The frozen specimens were fractured using a Denton apparatus at −100 °C and etched for 1 min. A replica was obtained by evaporating platinum-carbon. The replicas obtained from *Ulva*, *Euglena* and spinach were cleaned by subsequent immersion in 70 % sulfuric acid for 2 h, 10 % sulfuric acid for 5 min., a concentrated sodium hypochlorite solution for 30 min, and distilled water for 5 min. Presumably since *Tribonema* cells form rather long aggregates, the procedure mentioned is not successful for this species Therefore, in the case of *Tribonema* samples the following cleaning solutions were used: 4 N sodium hydroxide at 70 °C for 90 min; conc. sulfuric acid at 70 °C for 90 min, and distilled water at room temperature for 5 min.

The replicas were then transferred to copper grids and studied with either a Philips EM 200, an EM 201 or an EM 301 electron microscope

Particle-carrying areas of membrane faces were measured with a planimeter.

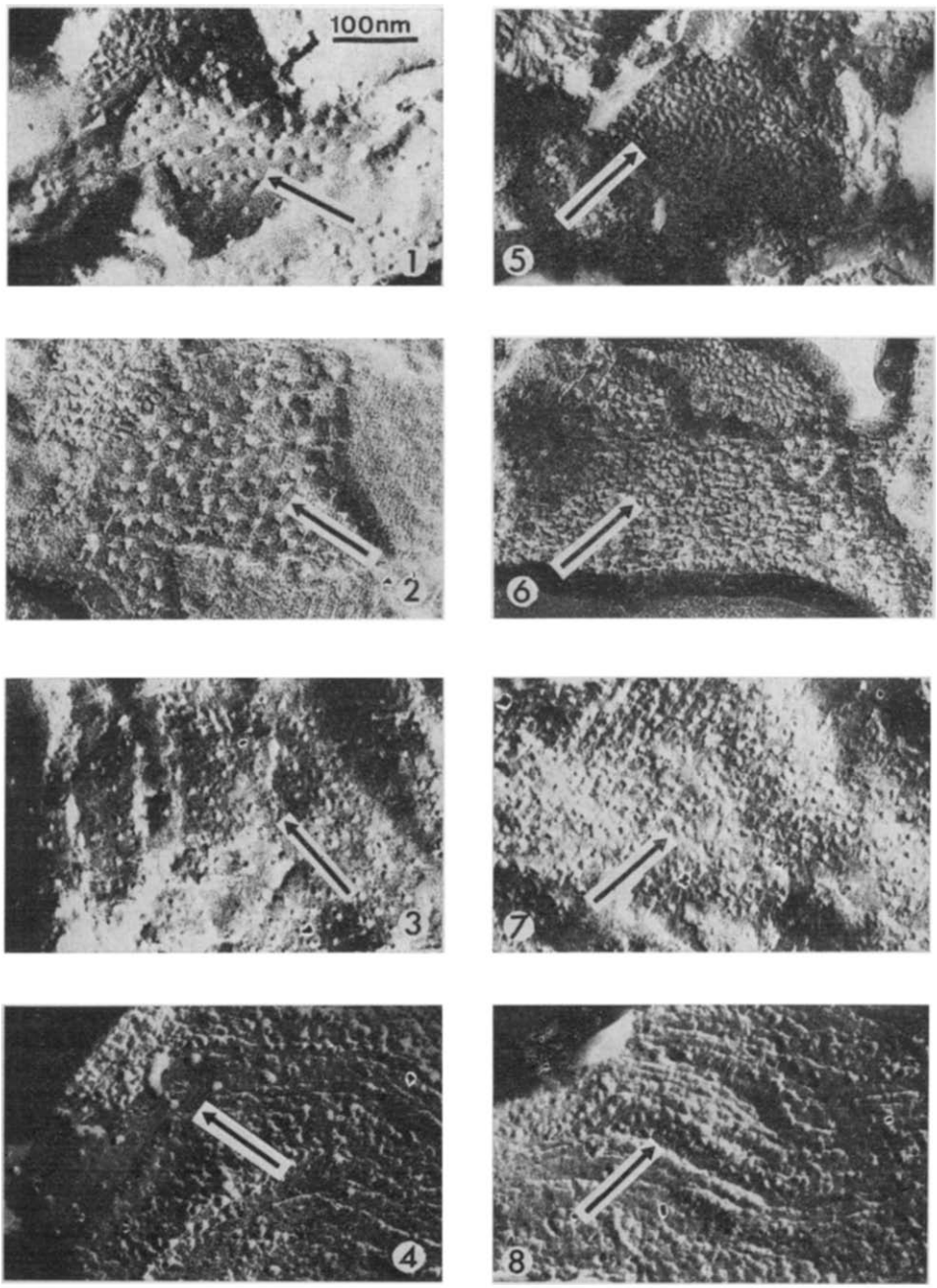

Fig 1 Examples of the studied intramembranous particles Large and small particles of *U. lactuca*, 1 and 5, of *S oleracea*, 2 and 6, of *E gracilis*, 3 and 7; as well as low-density (4) and high-density (8) particles of *T aequale* The magnification is the same for the various electron micrographs

Particle diameters were determined with a binocular microscope, BM-51-2, magnification about 9×, equipped with a micrometer grating (see ref. 16).

For various reasons [17] the magnification shown in the electron micrographs is inherently inaccurate to about 10 %. In exceptional cases the deviation may amount to 20 %.

Absorption measurements

Absorption spectra at 77 K were recorded in a Cary Model 14R spectrophotometer. Analyses of these spectra were made with 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 refs. 18–20. The analyses yielded seven components termed below: chlorophyll *b* and, chlorophylls *a*-640, 662, 670, 680, 685 and 695.

RESULTS

Usually the EF (exoplasmic fracture) and PF (protoplasmic fracture) surfaces [21], formerly termed B and C faces, carry large and small particles respectively. Examples of these particles from the studied species are shown in Fig. 1. The various data are summarized in Table I.

TABLE I

Diameters and population densities of intramembranous particles from chloroplasts, as well as relative amounts of the various pigment complexes, in four plant species. These amounts are expressed as percents of total area of the real absorption band between 710 nm and 620 nm. Numbers in parentheses indicate the numbers of particles measured or counted

Particle type	Determination	*Ulva lactuca*	*Euglena gracilis*	*Spinacia oleracea*	*Tribonema aequale*
Large particles or low population density	(a) diameter, (nm)	11 6±0 2 (279)	8 6±0.3 (86)	12.1±0.2 (238)	10 4±0 3 (49)
	(b) number of particles per 10^{-2} μm^2	17±1 (388)	19±1 (673)	15±1 (506)	10±3 (192)
Small particles or high population density	(c) diameter, (nm)	7 6±0 1 (201)	7 1±0 4 (57)	8 5±0 2 (175)	10 4±0.2 (137)
	(d) number of particles per 10^{-2} μm^2	50±2 (759)	70±2 (373)	54±2 (1789)	37±2 (1201)
Ratios	diameter (c)/(a)	0 66±0.01	0.83±0 05	0 70±0 02	1.00±0.03
	density (d)/(b)	2 9 ±0.2	3 7 ±0 2	3 6 ±0 3	3 7 ±1 1
Relative amounts of pigment complexes, %	Chlorophyll *b*	12 7	9 6	9.0	0
	Chlorophyll *a*-640	11.6	1.6	7.8	0
	Chlorophyll *a*-662	17 7	16.6	15 5	14 7
	Chlorophyll *a*-670	20.2	31 0	18.6	46.0
	Chlorophyll *a*-680	25 0	16.0	37.0	38 5
	Chlorophyll *a*-685	12.0	6 5	7 0	0
	Chlorophyll *a*-695	1.0	18 9	5 0	0 9

In *Tribonema*, particles of a single size occur. However, these particles are arranged at two clearly different population densities. According to Boardman et al [4, 5] the absence of chlorophyll *b*, as is the case with *Tribonema*, is correlated with the absence of large particles. When comparing the data from Table I it is obvious that the *Tribonema* particle size lies between those of the large and small particles of the others species studied. We therefore refrain from classifying the *Tribonema* particles as large or small.

Moreover, according to Schwelitz et al. [22] intramembranous particles of a single size, but occurring at two densities, are also observed in the chlorophyll *b*-carrying alga *E gracilis*. As shown in Table I, the sizes of particles located at both densities differ according to our measurements although not very significantly. For this reason we hesitate to consider the particles from this species to be of a single size and prefer to distinguish between large and small particles in *Euglena*. For *Ulva* and for spinach the occurrence of two kinds of particle is obvious. The frequency distribution of the sizes of both classes of particle is shown in Fig 2

As mentioned under Materials and Methods, magnification errors in electron micrographs may amount to about 20 % in extreme cases, but their exact estimation is impossible. The standard deviations given in columns (a)–(d) of Table I, refer only to measurements. The actual values of these deviations are therefore considerably larger. To circumvent this difficulty the diameter ratios of both kinds of particle, as well as the pertaining population densities, are presented. Since these particles are measured or counted in the same micrographs the magnification problem does not arise in this

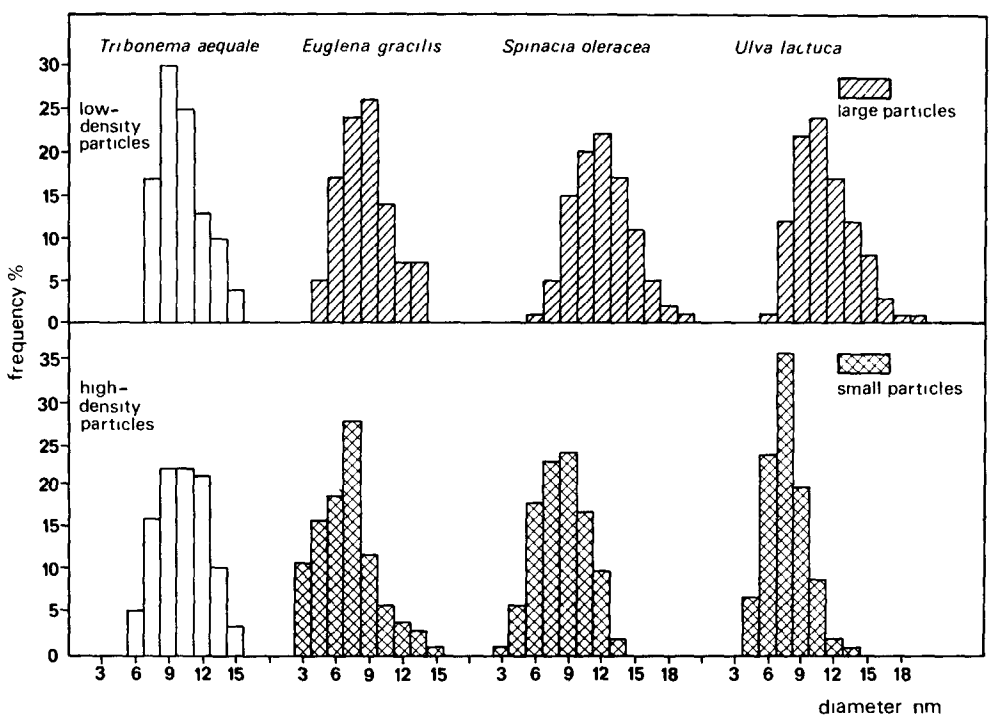

Fig 2. Frequency distribution of the sizes of the examined intramembranous particles

case, and the standard deviations of the ratios represent the actual values. The diameter ratios, then, confirm that in *Tribonema* only single-sized particles occur, whereas the remaining species contain both large and small particles

From Table I it can be concluded that in a species with intramembranous particles of a single size chlorophyll *b*, chlorophyll *a*-640 and chlorophyll *a*-685 are absent, whereas the remaining species contain large and small particles as well as all observed kinds of chlorophyll complexes However, no quantitative relationships between particle sizes and densities, nor relative amounts of pigment complexes, are observed.

DISCUSSION

Attention may be drawn to the fact that the mean diameters of large and small particles, found to be approx. 11 nm and approx 8 nm respectively in the present study, are smaller than those measured by several authors [16, 21, 23–27], who reported diameters of about 17 nm and 11 nm for large and small particles respectively The larger values for particle diameters from the same origin may be ascribed to differences in the platinum-carbon procedure. For instance, the particle size is possibly increased by impingement of carbon prior to the shadowing by platinum [28]. It may be added that the results of Pfeifhofer and Belton [29] agree with our measurements.

When the absence of chlorophyll *b*, chlorophyll *a*-640 and chlorophyll *a*-685 correlating with the occurrence of only single-sized intramembranous particles is considered along with a previous suggestion [20] about the presence of these forms in the same complex, it may well be that the absence of the carrier moiety of this complex is correlated with the absence of a second type of particle. It should be additionally remarked that Döring et al [30] and Leppink and Thomas [31] observed a correlation between the chlorophyll *a*-685 and chlorophyll *a*-640 bands Therefore it has been tentatively suggested [20] that these bands are due to one and the same chlorophyll *a* form, called chlorophyll *a*-685

The occurrence of a complex carrying both chlorophyll *b* and chlorophyll *a*, and its possible effect on lamellar structure, was proposed by Thornber and Highkin [11], who called it the light-harvesting chlorophyll-protein complex. Butler and Kitajima [32] also concluded the presence of such a complex from experiments on energy transfer

Since no correlation is observed between the relative amounts of the various chlorophyll forms and either the size or the population density of the particles, it may be concluded that the occurrence of the carrier moiety of the light-harvesting chlorophyll · protein complex, but not the relative amount of chlorophyll *b* or chlorophyll *a*, is engaged in the formation of one of the types of intramembranous particles Because of the intermediate size of the particles from the chlorophyll *b*-free alga *T aequale*, it cannot be concluded which type of particle is involved However, since according to the suggestion by Thornber and Highkin [11] the light-harvesting pigment complex may keep the photosynthetic lamellae in contact with each other, the type in question may represent the large particles If so, the conclusion of Boardman et al. [4, 5] that chlorophyll *b*-free species are devoid of large particles is correct.

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