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CHLOROPHYLL CONCENTRATIONS IN PLASTIDS OF DIFFERENT GROUPS OF PLANTS

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Introduction

In various groups of plants, the photosynthetic apparatus is differently built. The following structures are known to occur: grana-bearing or granulated chloroplasts, grana-free or lamellated chloroplasts, and free grana. For details, the reader may be referred to various extensive reviews of *e.g.* E. Weier (1938), Rabinowitch (1945), Granick (1949), Strugger (1951), T. E. Weier and Stocking (1952), Frey-Wyssling (1953), Straus (1953), Thomas (1955). The three types of plastids have one feature in common: the chlorophyll-bearing parts are lamellae.

In lamellated chloroplasts and in free grana these chlorophyll-bearing lamellae extend throughout the organelle. Fluorescence microscopy (P. Metzner (1937), Strugger (1950), Düvel and Mevius Jr (1952), Frey-Wyssling and Steimann (1953)) as well as evidence obtained with the Molisch reaction (H. Metzner (1952)) render it highly probable that in granulated chloroplasts the chlorophyll is restricted to the grana. By the aid of the latter technique it is possible to show that the pigment is most probably attached to the lamellae of the grana (Thomas, Post and Vertregt (1954)). Hodge, McLean and Mercer (1955) presented a scheme of the *Zea Mais* chloroplast in which they put the chlorophyll in the intra- as well as in intergranular lamellae. However, on account of the evidence mentioned above, the calculations in this study have been based on the view that, in granular chloroplasts, the chlorophyll is entirely concentrated at the intragranular lamellae. From a study of dichroism, of anomalous dispersion of birefringence, and of fluorescence polarisation Goedheer (to be published) concludes that this pigment occurs in a monolayer at the lamellar surface, and that its molecules are, though imperfectly, oriented. He calculates that in *Mougeotia* chloroplasts the lamellar area available per chlorophyll molecule is of the order of 250 Å². WOLKEN and SCHWERTZ (1953) found nearly the same value for *Euglena* and *Poteriochromonas* chloroplasts.

In various kinds of plastids the presence of a highly efficient energy transfer between chlorophyll molecules was found, *cf* DUYSENS (1952). As the plastid structure of these species may be widely different, this means that in all these different kinds of plastids, the chlorophyll concentration must, at least locally, be sufficiently high to enable such an energy transfer. For this reason it seems worthwhile, in both structural and functional respect, to examine chlorophyll concentrations in relation to lamellar area in a number of differing plastids. The results of such a study are presented here.

METHODS

The experiments were made with freshly collected material.

Counting of plastids. Leaves of the land plants *Spinacia oleracea, Hibiscus rosa-sinensis,* and *Tulipa* were squeezed in a tissue press, fig. 1, devised by A. N. van Straten. The juice was filtered over cotton wool.

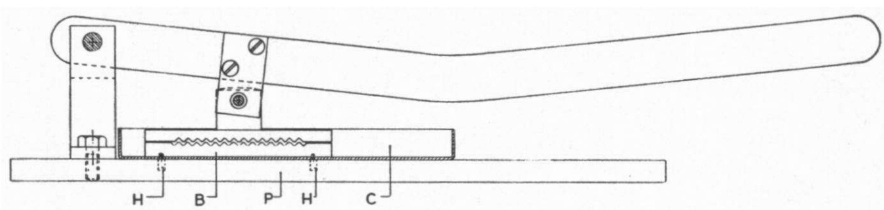

Fig. 1. Tissue press designed by A. N. VAN STRATEN. The lower press block (B) is fixed to the juice receptor (C). C is held in position by means of pins fitting in holes (H) in the basal plate (P). In this way the receptor can easily be removed.

The chloroplast concentration was determined by means of a counting chamber in samples that were put under the microscope. Because of the toughness of the leaves, in *Aspidistra elatior* the number of chloroplasts per cell, the number of cells per layer, and the number of cell layers was determined by means of the ordinary microscope; 1 cm³ of the leaf was cut out and used for chlorophyll estimation.

The chloroplasts of the aquatic plant *Elodea densa* are easily ruptured. Since the leaves consist of two cell layers it was possible to count the chloroplasts *in situ* as well. The mean area of both cells and leaves was measured, and the number of cells per leaf calculated. For chlorophyll estimation 200 leaves were used.

The green algae *Mougeotia* and *Spirogyra* contain a single chloroplast per cell. The cell volume was measured under the microscope. The sample to be analysed was dried with filter paper, and its volume was determined pycnometrically. In this way the number of chloroplasts in such a sample can be found.

In suspensions of the remaining objects the cell concentration, *cf* Table I, was estimated in samples by means of the counting chamber. The number of plastids was determined with the electron microscope.

Estimation of plastid volume and lamellar area. This was carried out by means of electron micrographs of both shadow-casted suspension preparations and sections (ELBERS, MINNAERT and THOMAS (to be published)). Necessarily the following assumptions and simplifications had to be made. First, it was assumed that the preparation technique caused no deformation. This may be nearly true of the sectioning procedure, but in the dried suspensions certainly some deformation will occur.

With cross-sections it is often difficult to state whether a pile of lamellae represents a whole granum or only part of a granum. Since this is of no interest in the present study, each more or less separate pile was regarded as a granum. For this reason the number of grana given in our table is presumably too high. However, as in this way the number of lamellae per granum will be reduced in the same measure, automatically a compensation is obtained.

In *Synechococcus* a single cup-shaped chloroplast of the lamellate type occurs. In the calculation its shape was considered to be a hollow cylinder closed at one end by a flat "bottom".

The mean number of windings of the *Spirogyra* chloroplasts studied here was 8. In ordinary micro-photographs the width of these chloroplasts was estimated at 1/30 of the length of a single winding. The pyrenoids were considered to occupy $\frac{1}{2}$ of the chloroplast area. For *Mougeotia* this value was estimated at $\frac{3}{4}$.

We are aware of the fact that these values are only rough approximations. They are all the more uncertain as it is not known whether the lamellae in the direct vicinity of the pyrenoids participate in the photosynthetic process or not, and if so, to what extent they do this, *cf.* STEINMANN (1952) and LEYON (1954).

The shape of *Rhodospirillum rubrum* made an estimation of the number of free grana rather difficult. The calculations were based on the assumption that the average bacterium is shaped as a spiral with one winding and with a constant diameter.

The suspensions for electron microscopical examination were prepared as described earlier (THOMAS, BUSTRAAN and PARIS (1952)). The sectioning technique developed by the third author, will be published in the near future (ELBERS, MINNAERT and THOMAS).

The quantitative estimation of chlorophyll was carried out according to ARNON (1949), that of bacteriochlorophyll after VAN NIEL and ARNOLD (1938–1939).

RESULTS

The results are summarised in Table I. As pointed out above, some assumptions and simplifications with regard to shape and structure of the plastids had to be made in order to make calculation possible. Therefore, the values of columns *k*, *l*, and *m* indicate merely the order of magnitude.

The area of lamellae from grana was determined in electron-micrographs of both dried suspensions and sections. The figures of

TABLE I

Chlorophyll density at lamellar surfaces in plastids from different origin

a	b	c	d	e	f	g	g′	h	i	j	k	l	m
Object	Type of plastid	chlorophyll content of a single plastid in gr. 10^{-12}	Plastid area in μ^2	Distance between centres of grana in a single layer in μ	Number of layers	Number of grana per chloroplast $\frac{df}{e^2}$	Number of free grana, or of grana-free chloroplasts per cell	Number of lamellae per granum, or grana-free chloroplast	Area of a single lamella in μ^2	Chlorophyll per μ^2 lamellar double layer area in gr. 10^{-16} $\frac{c}{g(g')hi}$	Chlorophyll per μ^2 lamellar monolayer area in gr. 10^{-16}	Number of chlorophyll molecules per μ^2 lamellar monolayer $.10^5$	Mean area of lamellar monolayer available per chlorophyll molecule $Å^2.10^2$
LAND PLANTS													
Spinacia oleracea	grana-bearing chloroplasts	1.9	56	0.55	6	1120	—	9	0.20	9.4	4.7	3.1	3.2
Hibiscus rosa sinensis	grana-bearing chloroplasts	1.(appr.)	54	0.58	5	800	—	15	0.10	8.3	4.2	2.7	3.6
Aspidistra elatior	grana-bearing chloroplasts	14.0	180	1.41	3	458	—	26	0.36	33.0	16.0	11.0	0.9
Tulipa spec.	grana-bearing chloroplasts	0.07	8.5	0.61	5	115	—	6	0.06	16.0	8.0	5.4	1.8
AQUATIC PLANTS													
Elodea densa	grana-bearing chloroplasts	2.0	71	0.50	4.5	1318	—	15	0.10	11.0	6.0	4.0	2.5
GREEN ALGAE													
Mougeotia spec.	grana-free chloroplasts	67.0	4420	—	—	—	1	170**)	1105.0	—	3.9	2.6	3.8
Spirogyra spec.	grana-free chloroplasts	340.0	9600	—	—	—	1	120**)	4800.0	—	6.0	4.0	2.5
BLUE-GREEN ALGAE													
Synechococcus cedrorum	grana-free chloroplasts	0.26	4.3	—	—	—	1	32**)	4.3	—	18.0	12.0	0.8
DIATOMS													
Nitzschia dissipata	grana-free chloroplasts	0.9 *)	10.2	—	—	—	2.5	60**)	10.2	—	5.9	4.0	2.5
BACTERIA													
Rhodospirillum rubrum	free grana	0.016 *)	0.02	—	—	—	34	7	0.02	29.0	14.0	10.0	1.0

* Chlorophyll content per cell. ** Monolayers.

column *i* refer to measurements made in the latter. Those obtained from suspensions surpass those of column *i* by a factor 2–3. Since deformation is expected to occur more readily in dried preparations than in sections, the values derived from the latter were preferred.

Table I shows that, even though both area and chlorophyll content of the various plastids, columns *c* and *d*, may vary with a factor 10^4, the lamellar area available per chlorophyll molecule, column *m*, proves to be everywhere of the same order of magnitude. In the mean it is a few times 100 Å^2.

Discussion

First, two general remarks may be made.

The number of grana per chloroplast, Table I column *g*, is unusually high. This is at least partly due to the above mentioned way of interpreting electron micrographs of sections. In addition, however, the sectioning technique seems to reveal that the number of grana per chloroplast is higher than it was previously supposed to be when the estimations were based on dried suspensions. It was also noticed that the dimensions measured in dried and in sectioned lamellae do not agree. For this reason it seems advisable to be very careful when drawing conclusions from electron micrographs of dried preparations.

The second remark deals with the nomenclature of chlorophyll-containing plastids. Because in electron microscopical preparations of dried suspensions no fundamental difference could be observed between lamellar chloroplasts and free grana it was suggested by one of us (Thomas (1955)) that the terms "granular chloroplast" and "lamellar chloroplast" might be discarded and replaced by the terms "chloroplast" and "granum" respectively. The application of the original terms seemed merely to rest on the size of the particles. In this way it was difficult to decide whether plastids of intermediate size, e.g. those of diatoms, should be called "chloroplasts" or "grana". However, when introduction of the sectioning technique and improvement of fixation methods enabled more accurate observation, it seemed possible to indicate distinct differences between lamellar chloroplasts and grana. These differences concern the way in which the lamellae are arranged.

In granulated chloroplasts intragranular lamellae consist of 70–100 Å thick double layers *cf.* Frey–Wyssling and Steinmann (1953), Finean, Sjöstrand and Steinmann (1953), Steinmann and Sjöstrand (1955) and Hodge, McLean and Mercer (1955). In the grana-free chloroplasts of *Fucus* and *Chlorella* Leyon and von Wettstein (1954), and Albertson and Leyon (1954) respectively observed that the about 50 Å thick lamellae are aggregated in sets of 4–8, thus forming laminae. The same may be seen in a figure, nr 7, of a cross-section of *Nitella* chloroplasts published by Mercer *et al.* (1955), while the width of the laminae in the "chromatoplasm" of *Phormidium* shown in a figure, nr 7, found in a paper by Niklowitz and Drews (1956), suggests that these laminae too possess this structure. In the present study a number of electron micrographs (Elbers, Minnaert and

THOMAS (to be published)) revealed that such structures also occur in the lamellated chloroplasts studied here.

These data suggest that there is a fundamental difference between grana and lamellated chloroplasts. In grana the lamellae are double layers which are not aggregated in sets, while in lamellated chloroplasts the lamellae seem to be monolayers and occur in sets of 4–8. If this is right, the plastids of the studied diatom should be called lamellated chloroplasts. At present it is still too early to propose a definite classification key. However, the earlier mentioned suggestion (THOMAS (1955)) seems to be contradicted by the observations given above and therefore is withdrawn.

NIKLOWITZ and DREWS (1955) prepared sections of *Rhodospirillum rubrum*. They observed lamellar structures which are absent in non-photosynthetic bacteria. The authors remarked that these structures may contain photosynthetic pigments. It was made highly probable by PARDEE, SCHACHMAN and STANIER (1952), SCHACHMAN, PARDEE and STANIER (1952) and THOMAS (1952) that in photosynthetic bacteria the bacteriochlorophyll is stored in grana. In the micrographs used in the present study the lamellated structures were also observed, and they are assumed to represent free grana. When the bacteriochlorophyll density at the lamellar surface is calculated on the base of this assumption, it proves to be of the same order as the chlorophyll density in other objects. Our assumption seems to find corroboration in this result.

It is evident from Table I that, though structure and dimensions of the various plastids widely vary, the mean area of lamellar monolayer available per chlorophyll molecule is of a constant order. DUYSENS (1952) came to the conclusion that energy transfer with an efficiency of 96 % between chlorophyll *a* and chlorophyll *b*, or between various bacteriochlorophyll types, requires a distance of 33 and 40 Å respectively between the centres of cubically arranged molecules. This distance corresponds with an area of $8.5 \cdot 10^2$ Å^2 and $12.6 \cdot 10^2$ Å^2 for, respectively, chlorophyll and bacteriochlorophyll molecules arranged in the same plane. The areas calculated in the present study, Table I column *m*, are smaller. This result indicates that in various kinds of plastids a highly efficient energy transfer is possible between chlorophyll molecules even when these molecules are regularly distributed over the lamellar surfaces.

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SUMMARY

The chlorophyll content of the plastids occurring in different group of plants was determined.

In the species studied here the number of chlorophyll molecules per lamellar area unit was found to be fairly constant.

These results, and also the possibility of a fundamental difference between lamellate chloroplasts and grana, were discussed.

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