ORIENTATION OF THE PIGMENT MOLECULES IN THE CHLOROPLAST

by

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INTRODUCTION

In the present investigation a certain orientation of the chlorophyll molecules and to a smaller extent of the carotenoids, was found to exist in the chloroplast.

Previously it was suggested by several authors (*cf.*[1]) that *in vivo* the photosynthetic pigments are oriented. In the well known scheme of HUBERT AND FREY-WYSSLING the porphin heads of the chlorophyll molecules were supposed to be parallel with the direction of the lamellae in the chloroplast (granum), the carotenoids perpendicular to them. If this is true, one may expect that absorption anisotropy—dichroism—will occur: light vibration in the plane of the chloroplast would be absorbed differently from light vibration perpendicular to it.

However, the experimental results diverge and do not allow a definite conclusion concerning the orientation of the chlorophyll molecules. MENKE[2] observed the occurrence of dichroism in weakly pigmented chloroplasts of *Closterium*. This author remarked that the effect may not be due to oriented molecules, but to the arrangement of submicroscopical pigmented layers alternating with transparent ones of different refractive index, a phenomenon which is called form dichroism.

FREY-WYSSLING AND WUHRMANN[3] failed to notice any dichroism, an observation which suggests a random distribution of the chlorophyll molecules.

In the retinal rods of the eye, a system resembling the chloroplast structure, SCHMITT[4] found an appreciable dichroism of the visual purple.

With small grana-bearing chloroplasts a high pigment concentration as well as diffraction and scattered light may make the demonstration of dichroism difficult.

This may also be true with the big lamellate chloroplasts when they are covered with small scattering particles. Thus further investigations were needed on both chloroplasts and pigments oriented *in vitro*. An indirect method was found for the determination of absorption anisotropy.

METHODS

Absorption measurements in monochromatic polarised light were done on chloroplasts of *Mougeotia* and *Funaria*.

Monochromatic light was obtained by the use of interference filters or a double monochromator. The absorption was measured by photometry or by the use of a Leitz microscope photometer. The accuracy of these methods was of the order of 5 %.

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Mougeotia contains big, plate-shaped lamellate chloroplasts of uniform, easily measurable width (length 40–120 μ, width 28 μ, thickness 4–6 μ).

Funaria was used for the study of grana-containing chloroplasts. Both plants could be studied in a unicellular layer, so measurements could be done without damaging the cells.

Apart from absorption measurements, an indirect method for the determination of absorption anisotropy is possible.

It follows from the classical theory of electro-magnetism that in an absorption band the refractive index as function of wavelength follows a special curve: the curve of anomal dispersion. Thus a certain change in refractive index is correlated with the occurrence of absorption; and so in an anisotropic medium, difference in refractive index in two directions perpendicular to each other (birefringence) is correlated with a difference in absorption in those directions (dichroism). Under certain conditions one can be calculated from the other.

The dispersion of birefringence was measured for the chloroplasts of *Mougeotia* and *Funaria* and also for some other species, using a Berek compensator of a one order range.

It may be remarked that the above mentioned investigators[2,5] found the dispersion of birefringence to be anomal in the neighbourhood of 680 mμ. They did not, however, apply these results to the determination of dichroism.

It was also possible to orient chlorophyll-a molecules at ammonia oleate interfaces. Here too, both anomal dispersion of birefringence and dichroism could be measured and so the theoretical relations between them could be checked. To estimate the effect of form dichroism, the difference in refractive index between the alternating layers in the chloroplast was diminished by change of osmotic pressure in a 6 % sugar solution. This results in a dehydration of the protein aqueous lamellae, and so increases their refractive index.

RESULTS

In *Mougeotia* chloroplasts absorption measurements in light of 680 mμ showed a weak dichroism. Light was absorbed 10–15% more when vibrating in the plane of the chloroplasts than in a plane perpendicular to it. For other wavelengths the phenomenon, if present, was beyond the limit of accuracy. Dichroism was not visible for those chloroplasts, which were covered to a large extent with scattering globules.

Qualitatively, a small dichroism was perceived also in chloroplasts of *Closterium*.

Confirming MENKE[2] we found optical anisotropy for absorption as well as refractive index only for chloroplasts standing on their small side.

The results of birefringence measurements for *Mougeotia* are shown in Fig. 1. The explanation is given in the legends. Theoretically, the anomal dispersion of birefringence passes through zero at the top of the absorption band. We measure, however, a superposition of three types of birefringence: anomal birefringence, form birefringence and birefringence due to oriented lipide molecules. At 680 mμ we measure only the sum of the latter two.

The structure of the chloroplast, consisting of lamellae of different refractive indices, gives rise to a negative form birefringence, which was accepted to be independent of wavelength in first approximation. According to WIENER's[6] formulae, a wavelength dependency of form birefringence should occur in this region. However, this phenomenon is a second order effect as calculation shows.

The positive lipide birefringence also may be assumed to be independent of wavelength in this region, as the lipide absorption occurs in the far ultra-violet.

From the corrected graphs the difference in absorption was computed in a direction parallel to and a direction perpendicular to the plane of the chloroplast.

A marked change in polarisation colour in white light can be seen during the development of the *Mougeotia* cells. During most of their lifetime the polarisation colour is green with negative birefringence. In "young" and "old" cells however, the colour is red with positive birefringence.

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As the figures show, this difference can be accounted for by a smaller contribution of the "form birefringence", the eye being very insensitive to light with a wavelength longer than 680 mμ. Dehydration in a 6% sugar solution results in a similar decrease of form birefringence. So it is possible that in "young" and "old" chloroplasts the protein

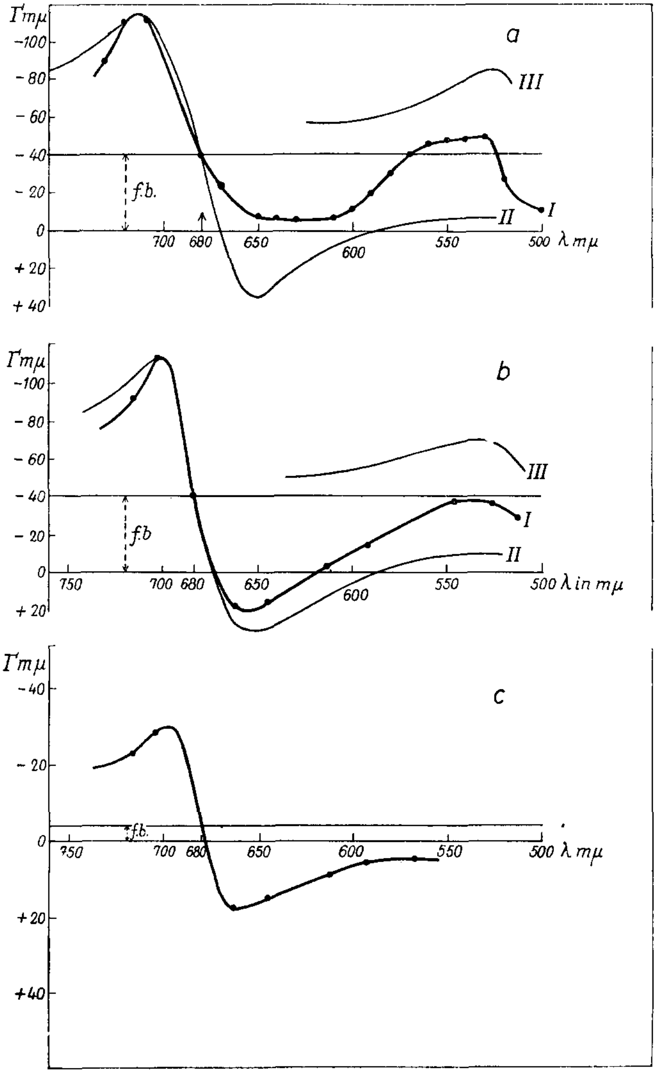

Fig. 1 (a). Anomal dispersion of birefringence of a fully developed *Mougeotia* chloroplast. The measured values (I) cover the computed ones (II) reasonably well in the region of the red absorption peak of chlorophyll. Between 680 and 620 mμ the measurements are rendered inaccurate due to high absorption and scattered light. The divergency between the computed values and the measured ones at wavelengths below 620 mμ can readily be explained in terms of orientation of the carotenoid molecules (III). In white light the colour of birefringence is green. Form-birefringence (f.b.) was found from the intersection of the measured curve and the vertical through 680 mμ. 1 (b). The same curves of a *Mougeotia* chloroplast not covered with scattering particles in a more early stage of development. Green birefringence colour, but after compensation of the green with the Berek-compensator, the chloroplast lights up red. 1 (c). Curve of anomal dispersion of a "young" *Mougeotia* chloroplast. In white light the colour of birefringence is red. This can be explained in terms of a smaller contribution of the form birefringence.

aqueous phase posesses a smaller water content than in other chloroplasts. The shape of the anomal dispersion curve does not change while decreasing the form birefringence.

It may be remarked that some types of algae, such as *Euglena viridis* nearly always show a "red" birefringence spectrum as shown in Fig. 1(c).

In *Funaria* chloroplasts, absorption measurements in light of 680 mμ could not reveal a dichroism exceeding the limit of accuracy. Here, however, the measured absorption is to large extent governed by diffraction and light scattering, as the ab-

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sorbing grana standing on their small side are smaller than the resolving power of the microscope.

Measurement of birefringence gives a curve of anomal dispersion with a shape as in Fig. 1 (b). Thus a certain absorption anisotropy exists in *Funaria* chloroplasts. An anomal dispersion of about the same magnitude has also been found for chloroplasts of *Tradescantia* and *Elodea*.

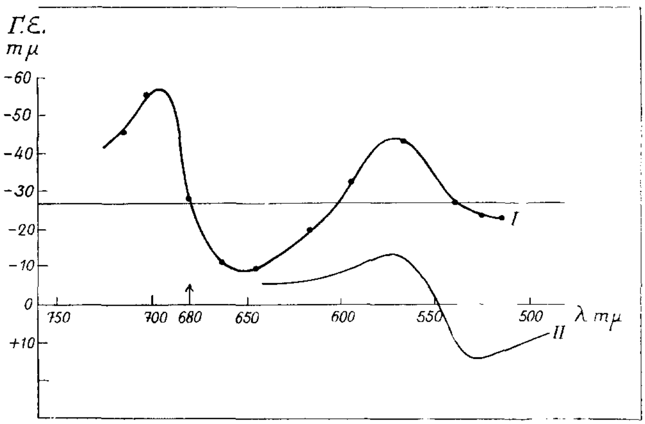

Fig. 2. Anomal dispersion curve of a *Mougeotia* chloroplast after vital staining with Rhodamine B (I). The colour of birefringence is yellow. The oriented dye molecules would show an anomal dispersion curve II. This is added to the original curve (see Fig. 1).

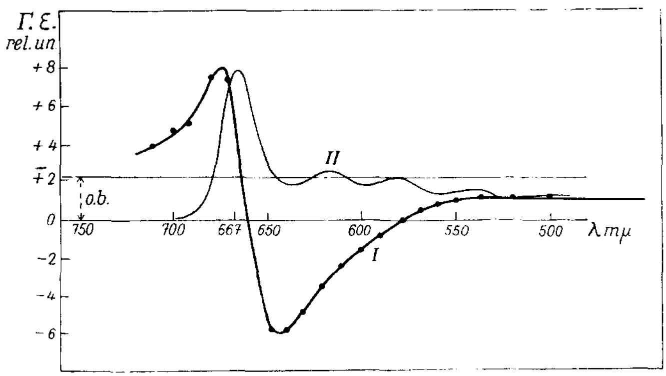

Fig. 3. Anomal dispersion of birefringence of chlorophyll a oriented at ammonium-oleate interfaces (I) and absorption spectrum of chlorophyll in ammonium-oleate (II). The value of birefringence due to the oriented oleate molecules (o.b.s) was found as given in Fig. 1 for form birefringence.

To check the calculations, measurements were made on chlorophyll oriented in a so called mesophase of ammonia oleate in a thin layer, where molecular orientation is to be expected.[7] The anomal dispersion of birefringence could be measured, (Fig. 3) while the dichroism could be determined at the top of the red chlorophyll band. Thus the measured and the computed dichroism could be compared. Both values proved to coincide satisfactorily.

An orientation of the lipide molecules can be demonstrated, as shown by MENKE[2], by the vital staining of chloroplasts with Rhodamine B.

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Then both dichroism and anomal dispersion of birefringence due to Rhodamine absorption at 550 mμ can be measured for *Mougeotia* chloroplasts (Fig. 2).

The fraction of the cross section which is stained by the dye can be calculated by measuring the absorption of the chloroplast, since the dimensions of the latter are known. The chloroplast is placed with its widest side facing the observer.

Calculations based on preliminary experiments show this fraction to be about 0.6. This method applied to the oriented part of the chlorophyll absorption yields a value of about 0.15 for the fraction of the pigmented part, or about one third of the remaining fraction available for the protein lamellae.

Also, the chlorophyll concentration in a *Mougeotia* chloroplast was calculated from the absorption of one chloroplast. We found a value of 0.01 *M* agreeing resonably well with values given by WOLKEN[8].

DISCUSSION

Since, from the above, it can be calculated that the absorption is higher in the plane of the chloroplast than in a plane perpendicular to it, the results demonstrate that the chlorophyll molecules are oriented in chloroplasts, though to a small degree.

For *Mougeotia* chloroplasts the ratio of absorption in either direction is 1.15. For *Funaria* and other small chloroplasts only the difference in absorption can be calculated. The measurement of absorption itself is inaccurate, due to shape and dimensions of the chloroplasts.

Form dichroism could be estimated according to the WIENER formulae, and was found to be small, with its sign opposite to that of the dichroism measured.

In chloroplasts, dichroism and anomal dispersion of birefringence have the same sign for chlorophyll and Rhodamine B. Their orientation in ammonium oleate or in myelin forms of lecithin, however, differs. Chlorophyll shows an orientation of the electric vector of the red absorption band in the plane of the molecules, whereas Rhodamine B shows an orientation perpendicular to them. Thus *in vivo* the plane areas of the chlorophyll molecules are preferentially oriented parallel to the lamellae of the chloroplast, the lipide molecules perpendicular to it.

The results shown in the figures indicate an orientation of carotenoid, in the same direction as the chlorophyll, *i.e.*, parallel to the lamellae. However, this orientation is much less pronounced than the chlorophyll orientation. Thus the picture of the carotenoid molecules perpendicular to the chlorophyll molecules does not hold.

Further, it may be remarked that with *Mougeotia* higher absorption differences were observed with more strongly coloured chloroplasts. So it seems probable that the ratio of the absorption values in question, the dichroism, is independent of concentration.

SUMMARY

Dichroism, absorption anisotropy, and anomal dispersion of birefringence were measured in the big lamellate chloroplasts of *Mougeotia*. The results of these measurements indicate a certain orientation of the chlorophyll molecules, and to a smaller extent, of the carotenoids in the chloroplast.

In species like *Funaria*, containing relatively small grana-bearing chloroplasts, absorption measurements in polarised light were too unreliable to detect a weak dichroism. However, the anomal dispersion of birefringence was found to be a common phenomenon for all chloroplasts measured.

It may be assumed from these results that a certain orientation of the pigment molecules occurs in all chloroplasts studied.

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RÉSUMÉ

La dichroism, anisotropie de l'absorption, a étudié avec les chloroplastes de *Mougeotia* et *Funaria*. Toutefois, grâce à la structure lamellaire des chloroplastes, l'observabilité de la phénomène en question est diminuée, par exemple en conséquence des effets de concentration forte et de diffusion de la lumière.

Néanmoins, comme on a observé une dispersion anomale chez la région spectrale du maximum d'absorption rouge de la chlorophylle, une méthode indirecte pourrait procurer des informations désirées.

Par rapport à la liaison de l'index de réfraction et du coëfficient d'absorption, on peut montrer l'existence d'une dichroisme faible.

Par conséquence, dans la chloroplaste les molecules de chlorophylle sont orientées imparfaitement, et à moins, aussi les caroténoides.

ZUSAMMENFASSUNG

Die Chloroplaste von *Mougeotia* und *Funaria* wurden untersucht auf das auftreten von Absorptionsanisotropie-dichroismus.

Die Komplizierte lamellare Structure macht es aber schwierig die Störende Effekte von z.b. Pigmentskonzentration und depolarisiertes Streulicht zu beseitigen.

Seit jedoch eine anomale Dispersion der Doppelbrechung im Gebiet des roten Absorptionsbandes von Chlorophyll observiert worden ist, gibt es eine Möglichkeit zur indirekte Bestimmung des Dichroismus.

Dies gründet sich auf die Zusammenhang von Refractions und Absorptions Koëffizienten.

In dieser Weise ist ein schwacher Dichroismus nach zu weisen. Also könnte gezeigt werden, das die Chlorophyllmolekülen im Chloroplast in Unvolstandiger Masze orientiert eingelagert sind, und, in eben geringere Masze, auch die Karotenoide.

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