MOLECULAR ORIENTATION IN QUANTASOMES

III. A Flow Dichroism Apparatus and Its Application to the Study of the Structure of Spinach Quantasomes

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ABSTRACT A new apparatus is described for measuring dichroism spectra with very high sensitivity for macromolecular structures oriented in a hydrodynamic gradient. The method has been used to explore the dichroism spectrum of quantasome aggregates isolated from spinach chloroplasts. The quantasome flow dichroism resembles qualitatively that observed previously using electric field orientation, in that a pigment absorbing at wavelengths longer than 680 mμ exhibits appreciably greater dichroism than those absorbing at shorter wavelengths. It is shown that the absorption oscillator for this long wavelength absorption lies parallel to the streamlines of the sheer gradient, which is assumed to be the direction in which the planes of the chloroplast lamellae are oriented.

INTRODUCTION

Recent studies in this laboratory of the electric dichroism spectrum of spinach quantasome aggregates (Sauer and Calvin, 1962) have prompted the construction of an apparatus for measuring the dichroism of suspensions of macromolecules oriented by a hydrodynamic flow or velocity gradient. With such a device it is possible to study suspensions containing moderate electrolyte concentrations. These are impossible to use in high electric fields because of the decrease of the field actually present in the conducting suspension and because of the heating resulting from the high current carried by the ionic medium.

A flow technique for orienting macromolecules has been selected because of its ready applicability to dilute aqueous suspensions containing added electrolytes. Whereas asymmetry of the electric polarizability or the presence of a dipole moment is a requirement for a particle to be oriented in an electric field, the corresponding requirement in the case of a flow gradient is the existence of geometric or shape asymmetry of the suspended particles. Many materials of interest, including quanta-

some aggregates, possess such asymmetry. The flow technique is not applicable to incompressible spherical particles.

The principal application of flow orientation for macromolecular systems has been in the study of their birefringence, or asymmetry of refractive index. The subject of flow birefringence has been reviewed extensively (Edsall, 1942; Scheraga and Signer, 1960), and the theory describing the hydrodynamic forces acting upon non-spherical suspended particles can be obtained by reference to these works. The study of the absorption asymmetry or dichroism of flow-oriented suspensions has been the subject of a much smaller portion of the literature. The most pertinent citations are work by Ruch (1951) using an apparatus having many features in common with that described in this paper; several studies using polarization spectrophotometry of samples flowing through a thin cuvette with stationary walls (Cavalieri, Rosenberg, and Rosoff, 1956; Bird, Parrish, and Blout, 1958; Lerman, 1963) or with moving walls (Zucker, Foster, and Miller, 1952) and one study in which a rotating cylinder cell has been adapted to a commercial spectrophotometer (Higashi, *et al.*, 1963; Wada and Kozawa, 1964).

The determination of the structure of the photochemically-active subunits of chloroplasts of green plants is a problem for which the orientation of chromophores is of particular interest. Washed chloroplast lamellae containing the chlorophyll and other plant pigments, exhibit a high level of activity for the Hill reaction in the light using ferricyanide (Park and Pon, 1961; 1963; Sauer and Park, 1964) or 2, 3′, 6-trichlorophenolindophenol (Sauer and Park, 1964) as oxidants. In the presence of added photosynthetic pyridine nucleotide reductase they are able to carry out the efficient reduction of nicotinamide adenosine dinucleotide phosphate coupled with the reduction of ascorbate (Sauer and Biggins, 1965). In the presence of soluble stroma substances from the chloroplast these lamellar fragments can support carbon dioxide fixation in the light.

Studies using electron microscopy show that the chloroplast lamellae are made up to subunits of 100 to 200 A dimensions (Park and Pon, 1961; Park and Biggins, 1964); these subunits have been designated quantasomes (Park, 1962). The "quantasomes" or quantasome aggregates of this study refer to washed chloroplast lamellar fragments containing 10 to 50 of these individual quantasome subunits in a roughly planar array.

METHODS

Preparation of Quantasomes. Chloroplasts isolated from *Spinacia oleracea* leaves were prepared by the procedure described by Park and Pon (1961). The chloroplasts were lysed for 30 minutes in 10^{-2} M potassium phosphate buffer (pH 7.5), centrifuged, resuspended in fresh 10^{-2} M buffer, and stored overnight at 0°C. This suspension was then sonicated for 90 seconds with a Raytheon sonic oscillator (Raytheon Co., Waltham, Massachusetts), centrifuged at 30,000 $\times$g for 10 minutes and the precipitate discarded. The supernatant was then centrifuged at 68,000 $\times$ g for 30 minutes and the

precipitated lamellar fragments resuspended in phosphate buffer (10^{-3} M, pH 7.5). The resulting suspension was stored overnight at 0°C, under argon, before use.

An alternative preparation used was made from lyophilized quantasome aggregates suspended in 2×10^{-3} M potassium phosphate buffer which was then diluted with an equal volume of a 1 per cent solution of methyl cellulose (Fisher Scientific, 4000 CP). The methyl cellulose solution was prepared by dissolving 5 gm of the dry powder in 250 ml of water at 85°C, adding an additional 250 ml of water and cooling to room temperature with occasional stirring. After the solution was cooled for 12 hours at 4°C, it became relatively clear, and remained stable stored at room temperature for several months. The viscosities of several concentrations of the methyl cellulose were measured relative to pure water using an Ostwald pipet, and the data gave a linear semilogarithmic dependence on concentration. The viscosity of the 0.5 per cent solution is 15 times that of water.

Absorption Spectra. Spectra were recorded using a Cary model 14 spectrophotometer with a scattered-transmission attachment and a slidewire giving full scale pen deflection for absorbance of 0.1 (Sauer and Park, 1964).

Flow Dichroism Apparatus. The equipment constructed to measure flow dichroism was designed to achieve (*a*) high sensitivity for the measurement of dichroic ratios close to unity, (*b*) photometric detection of the optical signals, (*c*) wide wavelength response, from 220 to 1000 $m\mu$, and (*d*) automatic wavelength scanning and recording of dichroism data. Most of these goals have already been achieved; some require further modifications of the equipment.

In this apparatus, a collimated beam of monochromatic light is passed vertically through an annular cell containing the sample under study. The outer wall of the annular cylinder cell can be rotated at speeds up to 2000 RPM, giving rise to a linear flow gradient (in absence of turbulence) in the radial direction across the annular space. The light beam is masked so that it passes through only a small sector (20°) of the circular cross-section of the rotor cell. Between the monochromatic light source and the rotor cell is a polarizer centered on the vertical axis passing through the sample sector above it, and mounted so that it can be spun about its vertical axis at constant angular velocity (here 20 RPS). This serves to give an angular modulation (at 40 seconds^{-1}) to the direction of polarization in the horizontal plane for the beam of light incident on the sample. Above the sample is a suitable photomultiplier detector for monitoring the transmitted intensity.

A sample that exhibits dichroism in the presence of a flow gradient will present a sector to the light path which behaves as a partial linear polarizer, with its direction of polarization fixed in the horizontal plane. Since the light incident on this sector has a direction of polarization which is rotating at 40 seconds^{-1}, the light intensity transmitted by the combination will be modulated at 40 seconds^{-1}, with an amplitude determined by the amount of dichroism present in the sample at that wavelength. The mathematical relationship between this modulated component of the transmitted intensity and the dichroic ratio will be discussed below.

The electrical output signal from the photomultiplier is sent through an electronic filter circuit to select that component of the over-all signal which has the 40 seconds^{-1} modulation. The filtered signal is then amplified and suitably displayed on a meter, recorder, or oscilloscope.

The instrument was constructed, with a number of modifications, using the chassis of a flow birefringence apparatus manufactured by the Rao Instrument Co., Brooklyn,

New York. The basic instrument, including details of the construction of the rotor cell, has been described in the literature (Edsall, Rich, and Goldstein, 1952). The modifications made to the optical system are diagrammed in Fig. 1. Two alternative sources of monochromatic radiation have been used. The first utilizes a grating monochromator (Bausch and Lomb, 500 mm, 35 A/mm, Bausch & Lomb Optical Co., New York) coupled to a suitable light source: a 500-watt tungsten projection lamp (General Electric type CZX) powered by a regulated filtered supply (Sorenson VMD 150-8, with extra capacitance added), a xenon lamp (Osram, 150 watt, XBO 150 W/1) powered by the same supply, or a hydrogen lamp (Bausch and Lomb, 100 watt).

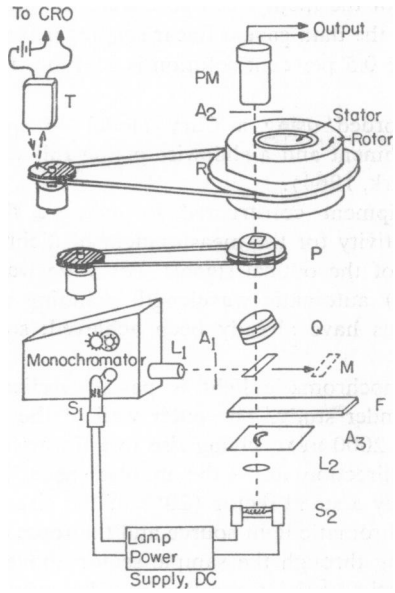

FIGURE 1 Diagram of optical components of the flow dichroism apparatus.

A synchronous motor (Hurst, 120 inch-oz) was coupled to the monochromator wavelength drum using one of a set of spur gears on the motor shaft and a wide gear, to allow for the linear travel, on the wavelength drum. In this fashion the wavelength could be scanned automatically at various speeds, most commonly 188 mμ per minute. The monochromatic light from the exit slit was collimated using quartz-fluorite achromats, L_1, (Bausch and Lomb, 33-86-53), passed through a defining aperture, A_1, and deflected vertically by a front-surface aluminized mirror, M. The light at this point is partially linearly polarized owing to the properties of the monochromator optics, *etc.* A polarization shim is introduced at Q. This shim consists of a pile of 3 to 6 fused silica flats mounted in such a way that they can be tilted to an adjustable angle to the light beam and can be rotated about the vertical axis. The device is quite comparable to the well known pile-of-plates polarizer. The two adjustable angles are set so as to effectively depolarize the light by reflecting out the excess polarization of the incident beam. The optimum depolarization for a given orientation of Q occurs at only one wavelength of light. Owing to the strong wavelength dispersion of the extent of polarization of light from the grating monochromator, a severe limitation is placed on the ability to measure

dichroic ratio spectra sensitively over wavelength ranges even as small as 50 to 100 mμ. This results from the method of detection of dichroism using the rotating polarizer. When the light incident on the sample rotor cell is partially polarized a background signal is obtained and must be subtracted from the apparent dichroism signal.

The alternative source of monochromatic radiation, suitable only in the visible and near infrared spectral regions, has the advantage of providing a beam with significantly less partial polarization than that from the grating monochromator. This arrangement is shown at the bottom of Fig. 1. The light from a small high-intensity tungsten lamp, S_2, (General Electric 1958, operated at 5 amps, 28 volts DC) is collimated using the achromatic lens, L_2, of the Rao apparatus, and is passed through a 2 mm wide aperture A_2 in the shape of the rotor cell arc. This beam is made monochromatic using an interference wedge, F (Schott Verlauffilter, VERIL S-200; obtained from Fish-Schurman Corp., New Rochelle, New York). The wavelength transmitted at each position of the filter varies linearly from 740 mμ at one end to 400 mμ at a position 15 cm away. The band halfwidth at any position is about 14 mμ and the dispersion is 2.42 mμ/mm. Since all the optical surfaces are very nearly normal to the light beam, no significant polarization is introduced using this type of "monochromator." Some residual polarization remains from the lamp bulb and the collimating lens; however, this is much smaller than that obtained from the grating monochromator. The interference wedge is mounted on a lathe-bed type of carriage. A synchronous motor driving a lead screw causes it to advance horizontally at a constant velocity across the vertical beam. In this fashion the wavelength range is scanned automatically at a rate of 74 mμ/minute. When this source of monochromatic radiation is used, the mirror M is removed from the light path.

The rotating polarizer, P, is mounted in a pair of precision ball bearings on a vertical axis and the frame is rigidly clamped to the round optical support bar of the Rao apparatus. A pulley is fixed coaxially between the two bearings and a synchronous motor (Bodine, 1800 RPM) is used to rotate the polarizer at constant velocity by means of a large diameter O-ring. The polarizing elements used are a Glan-Thompson prism or a Glan prism (Karl Lambrecht, Chicago, Illinois), but an adapter allows the use of a Polaroid disk (type HN-38) or a fused silica plate with a coating which polarizes in the ultraviolet (Polacoat Corp., Blue Ash, Ohio; types 105 or PL 53) (McDermott and Novick, 1961).

The design of the rotor cell has been described (Edsall, Rich, and Goldstein, 1952). A stator with outside diameter of 24.38 mm and a rotor with inside diameter of 25.40 mm were used, producing an annular gap of 0.51 mm. The only modification made is the use of top and bottom windows cut from plane, polished, fused silica plate to permit observations in the ultraviolet. The rotor speed was measured using a photoelectric tachometer.

The photometric detection system consists of a photomultiplier (RCA 7326 for the red; Du Mont 7664 for ultraviolet) with a plane end-window to avoid unwanted polarizations. The photomultiplier is carefully shock-mounted on a wood support frame separate from that of the Rao apparatus in order to prevent mechanical interference from the drive motors. The photomultiplier output is fed into both a Tektronix oscilloscope (Tektronix Inc., Portland, Oregon) (Model 550 with Type D preamplifier; second beam used for angular velocity tachometer signal) and a Hewlitt-Packard Model 302A wave analyzer, as shown in Fig. 2. The wave analyzer is tuned to 40 CPS in order to monitor the signal resulting from the rotating polarizer. The gain is set to an appropriate value and the output recorded using a Leeds and Northrup 0 to 10 mv recording potentiometer,

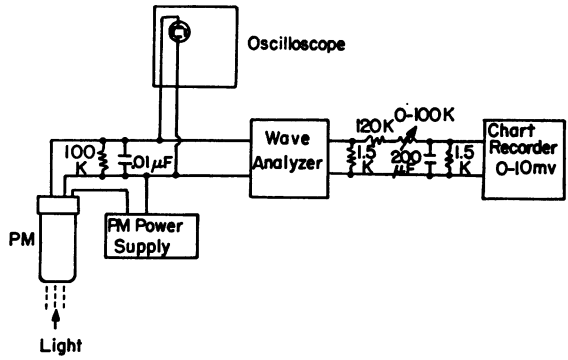

FIGURE 2 Electronic circuit for photometric flow dichroism measurements.

coupled through an appropriate impedance matching element. Dichroism spectra are recorded by setting the appropriate monochromator drive into motion and recording the output signal as a function of time.

Operations. Measurements of flow dichroism were made on suspensions of quantasome aggregates (spinach chloroplast lamellar fragments) exhibiting a low intrinsic dichroism. Samples were diluted to a concentration sufficiently low that the transmission in the wavelength region of interest was between 10 and 90 per cent in the 4.5 cm path-length rotor sample cell. Rapid evacuation and shaking of the suspension just prior to filling the rotor cell effectively prevented the subsequent formation of gas bubbles in the light path. The degassed sample solution was injected into the bottom of the rotor cell by means of a hypodermic syringe and long needle, as is described elsewhere (Edsall, Rich, and Goldstein, 1952).

The determination of dichroic ratio spectra requires several independent measurements. For dichroic ratios near unity a method analogous to that derived previously in connection with electric dichroism studies is used (Sauer and Calvin, 1962). It can readily be shown that under the conditions of the present study

$$D - 1 = \frac{\Delta I_{trans}}{2.3 I_{trans} A}$$

where ΔI_{trans} is measured by the amplitude (peak to peak) of the 40 CPS signal from the photomultiplier (less the background signal), I_{trans} is the average DC level of the photomultiplier signal, A is the absorbance of the sample in the 4.5 cm path length, and D is the dichroic ratio. Experimentally, it was more convenient to determine I_{trans} as a function of wavelength by inserting a fixed polarizer between the rotor cell, R, and the photomultiplier aperture, A_2. With the lower polarizer rotating and the rotor cell stopped, the transmitted light intensity is fully modulated at 40 CPS. In this fashion a signal, T, proportional to I_{trans} can be recorded as a function of wavelength. The magnitude of T must be increased by 10 per cent to account for the decrease in transmission caused by the added fixed polarizer for light polarized along its axis of maximum transmission. The value of ΔI_{trans} at each wavelength is determined by subtracting a background signal, b, obtained with the rotor cell fixed and the polarizer, P, rotating from the dichroism signal, d, obtained with both the rotor cell and the polarizer, P, rotating. The ratio $\Delta I_{trans}/I_{trans}$

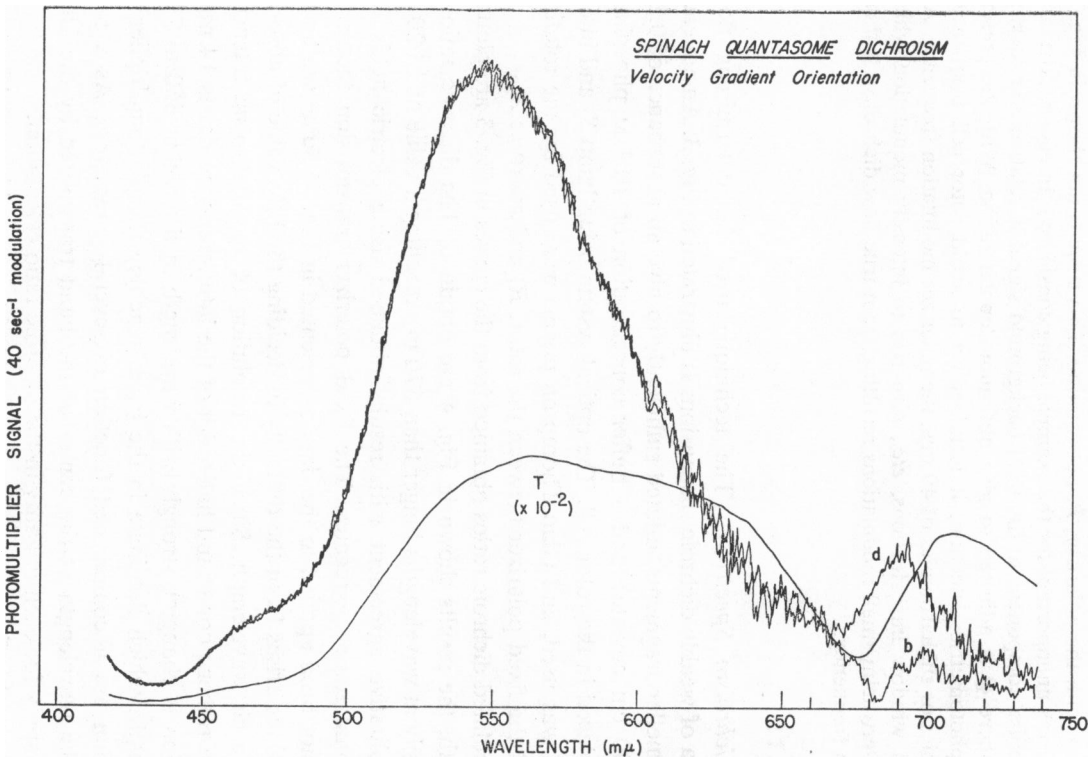

FIGURE 3 Flow dichroism of spinach quantasomes. Spinach quantasome aggregates (A_{678} = 0.97 for 4.5 cm path) in 10^{-5} M phosphate, pH 7.4 oriented in a flow gradient of 3500 seconds^{-1}. Curve *d:* modulated signal (40 seconds^{-1}) with rotor cell at 22.8 RPS and polarizer, *P*, rotating; curve *b:* modulated signal with rotor cell fixed and polarizer, *P*, rotating; curve *T:* modulated signal ($\times 10^{-2}$) with supplementary fixed Glan-Thompson polarizer and polarizer, *P*, rotating.

in the equation above is equal at each wavelength to the ratio $(d - b)/1.10\ T$, since the wavelength-dependent instrument parameters cancel in this latter ratio. Attention must be paid to the relative signs of d and b, since the sign of b, at least, will be opposite on either side of the wavelength at which Q is adjusted to null. This point will be discussed further below. Another component of the background is the noise, n, inherent in the signal at 40 CPS passed by the wave analyzer. This noise level is determined by repeating the above scan now with the polarizer, P, not rotating. This trace is not shown in Fig. 3, as its contribution was unimportant for the example being considered. In general, the contribution of this noise component to the total background signal is relatively greatest at the wavelength where Q is adjusted to give optimum depolarization. With the present apparatus using photometric detection, it is necessary to avoid rotor cell frequencies which are harmonics or subharmonics of 40 CPS, the polarizer modulation frequency. Air bubbles, wall and window imperfections, *etc.*, give rise to intensity modulation which can completely overwhelm small modulations resulting from true flow dichroism if there is a coincidence in frequency.

RESULTS

Flow Dichroism Spectrum. The technique used for determining flow dichroism spectra of weakly dichroic suspensions is illustrated in Fig. 3. An aqueous suspension of lamellar fragments, diluted sufficiently to give an absorbance of 0.21 at 678 mμ (1.00 cm cuvette) and a buffer concentration of 10^{-3} M phosphate (pH 7.4), was placed in the rotor cell. The optical system using lamp S_2 and interference wedge F was used, and Glan-Thompson prisms were used for the rotating polarizer, P, and the fixed polarizer between the rotor, R, and aperture A.[2]

When the calculated dichroic ratios obtained from the curves in Fig. 3 are plotted *versus* wavelength, the results shown in Fig. 4 are obtained. The dichroic ratio is seen to rise sharply at wavelengths longer than 670 mμ reaching a value of 1.020 at 700 mμ, in qualitative agreement with results obtained using electric fields for orienting the quantasome aggregates. The fixed polarizer transmission factor of about 1.10 has not been applied to the data represented in Fig. 4. Some scatter in the calculated values arises from the difficulty in deciding the best value of absorbance to use at each wavelength. Since the resolution of the dichroism intensity spectrum may be rather poor (band halfwidth of the interference wedge is 14 mμ) and the absorption is changing strongly with wavelength in the region 660 to 719 mμ, the wavelengths which dominate in the beam reaching the photomultiplier at each filter position are a complicated function of several parameters. As a first approximation the wavelength at the center of the band transmitted by the filter was assumed to apply for each quantity in the dichroic ratio calculation.

The magnitude of the dichroic ratio increment (D-1) reported in Fig. 4 is about 15 times smaller than that observed using electric orientation (Sauer and Calvin, 1962). There is undoubtedly a different relationship between the "optical" axes of the particles and the orienting force for these two techniques, and some difference in measured dichroic ratios can be expected to arise from this source. Nevertheless,

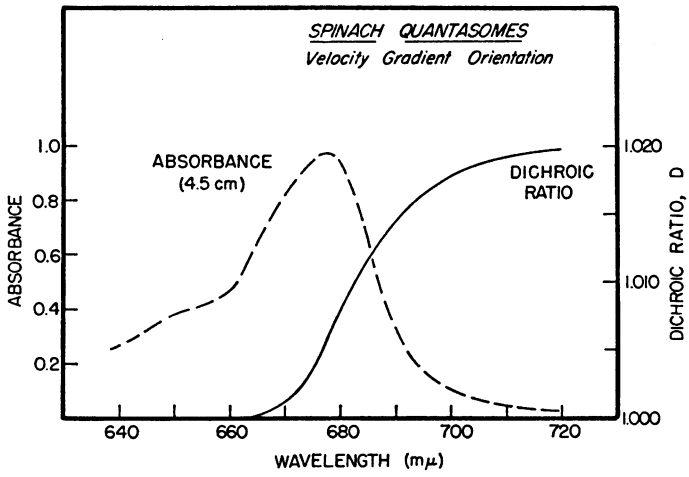

FIGURE 4 Dichroism spectrum of spinach quantasome aggregates. Solid curve: dichroic ratio versus wavelength for aqueous buffer suspension in velocity gradient $G = 3500$ seconds^{-1}. Dashed curve: absorption spectrum of quantasome suspension calculated for a 4.5 cm path length.

there is good evidence that much of the difference arises from fractional orientation of the particles in the hydrodynamic gradient. Experimental problems with the present apparatus make it difficult to explore the relationship of dichroic ratio *versus* velocity gradient for the aqueous quantasome suspensions. The sources of this difficulty are being investigated with the hope of improvement of performance. They arise principally from the small signal-to-noise ratio, which makes the use of a narrower rotor gap unfeasable, and the existence of the rotor frequency harmonic interference, which makes studies at somewhat lower frequencies impossible.

A different approach to the determination of the extent of orientation has been made by increasing the viscosity of the solvent phase through addition of a suitable inert substance. Materials such as glycerin or polyethyleneglycol are unsatisfactory because of their ability to extract pigment molecules from chloroplast lamellae, as measured by shifts in the visible absorption spectrum (Sauer, unpublished data). Methyl cellulose was found not to produce any apparent effect on absorption properties at concentrations up to 1.0 per cent. The addition of 0.5 per cent methyl cellulose to a buffered suspension of lyophilized quantasomes apparently greatly increased the susceptibility of the quantasomes to orientation, since the dichroic ratio increments of such suspensions measured at velocity gradients of 1500 seconds^{-1} were 10 times the values at the same wavelengths shown in Fig. 4. This gradient was observed to be well above that required to saturate orientation in the system. Furthermore, the observed dichroic ratio of 1.20 at wavelengths longer than 700mμ for the quanta-

somes in 0.5 per cent methyl cellulose is in reasonable agreement with the values observed using electric field orientation (Sauer and Calvin, 1962).

Absorption Oscillator Orientation. An additional piece of information is available from the flow dichroism measurements; namely, the direction of the absorption oscillator with respect to a particle-fixed axis system. The quantasome aggregates can be best characterized in terms of an axis normal to the plane of the lamellae from which they were disrupted. Since the shortest dimension of the disk-like structures is along this axis, it will tend to lie perpendicular to the stream lines in the flowing system. For samples exhibiting large flow dichroism effects it is sufficient to measure the transmitted intensity first with the polarizer oriented with its polarizing axis tangential to the annulus of the rotor cell and then with the polarizer oriented radially. The results will tell immediately the preferred direction of orientation of the absorption oscillator and allow it to be related to the particle axis. For small dichroic increments, such DC measurements are unreliable owing to the high noise level and possible small systematic errors. A way around this difficulty is presented through consideration of the properties of the polarization shim, Q.

The properties of the polarization shim, Q, will be described in terms of the diagram shown in Fig. 5. Space-fixed axes X and Y are taken to lie in the horizontal plane, with X parallel to the tangent of the circumference of the rotor cell and Y along its radius, *i.e.* passing through the optical support rod of the apparatus. The Z axis is in the vertical direction along the light path. Consider the optical shim, Q, to be at the center of the coordinate system. The normal, ON, to the fused silica plates of Q is at an angle ϕ with respect to the Z axis and its projection on the XY plane is at an angle θ from the Y axis. If unpolarized light is incident on the shim from below, then the part of the light reflected at Q will be completely polarized with its electric vector lying in a direction parallel to the intersection OM of the

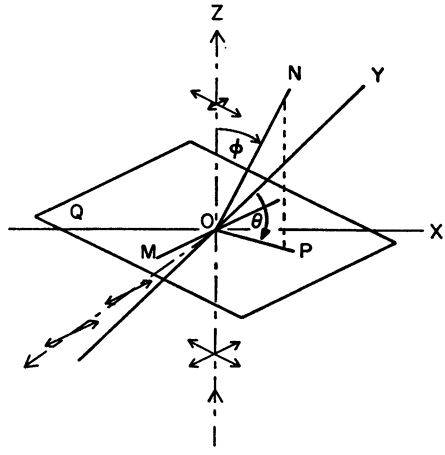

FIGURE 5 Polarization produced by a fused silica plate.

fused silica plates with the horizontal plane. The transmitted light will then be partially polarized, with its largest electric vector component lying in the plane containing the angle *ZON*. On the other hand if the incident light is partially plane polarized, the excess polarization can be removed by reflection by choosing appropriate values for ϕ and θ. To depolarize the beam at 700mμ in the present study using the interference wedge monochromator, ϕ is about 30° for three plates in Q, and θ is about about 100° measured clockwise from above, as shown in Fig. 5. At shorter wavelengths the transmitted beam tends to have excess polarization in the *ZON* plane (tangential) and at longer wavelengths the excess polarization is perpendicular to this plane (radial).

If the suspension in the rotor cell exhibits dichroism, then the net polarization of the beam incident on the photomultiplier will be the vector sum of the effect of Q and the effect of the oriented sample at each wavelength. The effect of Q can be altered in a predictable fashion by slightly altering either ϕ or θ. By observing whether the signal at the photomultiplier is increased or decreased by a given operation on Q, the preferred orientation of the absorption oscillators in the flowing suspension can be readily deduced. In this manner it was determined that the absorption oscillators responsible for flow dichroism of quantasome aggregates lie in the tangential direction, or along the flow lines, throughout the visible region of the spectrum. From this we can further deduce that the absorption oscillators tend to lie in the lamellar planes. This is consistent with the observations of Olsen, Butler, and Jennings (1962) on the polarized absorption of chloroplasts observed in the microscope using 700 mμ radiation. In the present study, however, any effects of form dichroism will be absent or greatly reduced.

DISCUSSION

The probable association of the long wavelength chlorophyll giving rise to marked dichroism with the photochemically active pigment P_{700} (Kok, 1956, 1961; Kok and Hoch, 1961) has been discussed previously (Sauer and Calvin, 1962). A comparison of the electric dichroism spectrum of the latter paper with the flow dichroism spectrum of this study demonstrates that the presence of the high electric field had no untoward effect on the intrinsic optical properties of the quantasome aggregates. Furthermore, the demonstration that the P_{700} absorption oscillator tends to lie parallel to the lamellar planes confirms the previous conclusion that these planes are preferentially oriented parallel to an applied electric field.

The curves of Figs. 3 and 4 suggest that the dichroic ratio for quantasome aggregates is 1.000 over the wavelength range 430 to 660 mμ. This is in contrast with the results of the electric dichroism measurements, which yielded a positive dichroic ratio somewhat greater than unity throughout this range. The flow dichroism studies in the presence of 0.5 per cent methyl cellulose, where stronger orientation is achieved, did give small positive dichroic ratio increments throughout this region.

These results were somewhat variable and it is felt that further work is needed before we fully understand the system with added methyl cellulose. In any event, we have not so far been able to observe any reproducible features corresponding to the dichroism maximum near 520 mμ which was exhibited in the electric dichroism spectrum. Further studies of this region and of the near ultraviolet dichroism spectrum are under way.

The author wishes to thank Professor Howard K. Schachman for the generous loan of his flow birefringence apparatus. For the purposes of studying flow dichroism it has undergone extensive modifications, of which he is only partly aware. Mr. John Biggins of this laboratory kindly provided some of the preparations of chloroplasts and chloroplast subunits used in this study. The author is indebted to the United States Public Health Service for a postdoctoral fellowship held during the early portion of this work. The research described in this paper was sponsored in part by the United States Atomic Energy Commission.

Received for publication, April 18, 1964.

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