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ORIENTATION OF THE HEMES OF HIGH POTENTIAL CYTOCHROMES RELATIVE TO PHOTOSYNTHETIC MEMBRANES, AS SHOWN BY THE LINEAR DICHROISM OF ORIENTED PREPARATIONS

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Summary

The orientations of high potential cytochromes with respect to photosynthetic membranes was investigated in spinach chloroplasts and in *Rhodopseudomonas viridis*. The general approach consists in detection with polarized light of photoinduced absorbance changes related to the oxidation of the cytochromes. The orientation of cytochrome *c*-558 was measured at room temperature in chromatophores and whole cells of *Rps. viridis*, oriented on glass slides and in a magnetic field, respectively. The orientation of cytochrome *b*-559 of green plants was detected at 77 K in magnetically oriented chloroplasts. In both cases the dichroic ratio for the α band shows that the heme plane makes an angle greater than 35°C with the membrane plane. Moreover, the dichroic ratio is not constant throughout the α and β bands, for both cytochrome *c*-558 and *b*-559. Linear dichroism spectra of oriented pure horse heart cytochrome *c* and cytochrome c_2 of *Rhodopseudomonas sphaeroides* in stretched polyvinyl alcohol films show that the variations of the dichroic ratio in the α and β bands can be explained by the occurrence of *x*- and *y*-polarized transitions absorbing at slightly different wavelengths.

Introduction

The roles of cytochromes as electron carriers in photosynthetic and respiratory systems have been recognized for many years. In bacterial photosynthesis

a cytochrome of the *c* type is the immediate electron donor to photooxidized bacteriochlorophyll (P^+) in the reaction center [1]. At room temperature cytochrome *c* reduces P^+ within less than a millisecond [2] and thus prevents the occurrence of a wasteful back reaction between the photochemically displaced electron and P^+.

In contrast to the extensive knowledge of the thermodynamics and kinetics of cytochromes *c* in bacterial photosynthesis [3], information concerning their location and orientation with respect to the membrane is fragmentary. Direct evidence of localization of cytochrome c_2 in *Rhodopseudomonas sphaeroides* and *Rhodopseudomonas capsulata* has come from the immunological study of Prince et al. [4]. More recently, some information on the orientations of cytochrome hemes in *Chromatium vinosum* and *Rhodopseudomonas viridis* with respect to the membrane plane was obtained by Tiede and coworkers [5,6] from EPR studies of air dried chromatophore films. It was found that the low potential cytochrome *c*-553 hemes lie parallel to the membrane plane in *C. vinosum*, whereas for the high potential cytochromes *c*-555 in *C. vinosum* and *c*-558 in *Rps. viridis*, the heme planes are roughly perpendicular to the membrane plane.

In this report we describe the orientation of cytochrome hemes with respect to the membrane plane in both green plants and photosynthetic bacteria, as determined from the linear dichroism of optical transitions in oriented samples. The orientation of the α band transition of cytochrome *b*-559 of green plants was determined by measuring the linear dichroism of the absorbance changes related to its photooxidation at 77 K, in magnetically oriented chloroplasts. We also measured the linear dichroism of absorbance and absorbance changes related to the high potential cytochrome *c*-558, in the α, β and γ bands, using oriented whole cells and chromatophores of *Rps. viridis* at room temperature. For comparison we studied the polarization of optical transitions in the α and β bands of oxidized and reduced horse heart cytochrome *c*, and of cytochrome c_2 purified from *Rps. sphaeroides*, in stretched polyvinyl alcohol films.

Materials and Methods

Rps. viridis was grown, and chromatophores prepared from the cells, as described earlier [7]. The chromatophores, suspended in 5—10 mM Tris · HCl (pH 7.5), were treated with approximately 1 mM 2,3,5,6-tetramethyl-*p*-phenylenediamine (diaminodurene) prior to drying them onto glass slides. This assured that the cytochrome *c*-558 in the dried films was in its reduced form, able to show light induced oxidation. The cytochrome *c*-552 remained oxidized under this treatment, and showed no light induced changes in the dried films.

Absorption and absorbance difference spectra of *Rps. viridis* preparations were recorded with a home-made split-beam spectrometer coupled to a Tracor-Northern TN-1500 Signal Averager and with a Cary 14R Spectrophotometer. Light-induced absorbance changes were measured with a single beam spectrometer, a modification of a split-beam instrument described elsewhere [8]. The output was received by a Tracor-Northern NS-575A Signal Analyzer. The latter two spectrometers were fitted with Glan-Thompson polarizers in their measuring beams. The slide bearing dried chromatophores was placed so that the axis

of the measuring beam made an angle of 60° with the normal to the slide, and the electric vector of vertically polarized light was in the plane of the slide [9]. Within the film of chromatophores the angle was estimated * to be 40° rather than 60°, due to refraction. Actinic light was provided by a saturating xenon flash with a decay half time of 0.1 ms, filtered through a broad 1000 nm interference filter. The measuring beam passed through a Corning 4-96 (blue) filter placed between the sample and the detector.

For orientation studies in a magnetic field, whole cells were suspended in 6.6 mM Tris · HCl, pH 7.8, and 33% glycerol. A magnetic field of 20—24 kgauss was applied as described by Paillotin et al. [10]. Actinic light was provided from the top of the cuvette by a dye laser, Electrophotonics Ltd. Model 23, operated at 600 nm. A Corning 4-96 filter was used in the measuring beam. The measuring beam was passed through a Rochon polarizer so that the electric vector of vertically polarized light was perpendicular to the lines of the magnetic field, while the horizontal polarization was parallel to the magnetic field. Comparison with other oriented preparations indicates that the cells became oriented with the majority of their internal membranes perpendicular to the magnetic field. All experiments with whole cells of *Rps. viridis* were made at room temperature with no electron donor added.

Spinach chloroplasts and barley etioplasts were prepared [11], and magnetically oriented suspensions of chloroplasts trapped at low temperature were obtained as described previously [12]. Absolute absorption spectra were recorded in the 535—565 nm region with a double beam spectrophotometer (Perkin-Elmer Model 356) operated in the split beam configuration. Both sample and reference beams passed through polarizing sheets (Polaroid HN37). Again, with horizontal polarization the electric vector was parallel to the magnetic field and predominantly perpendicular to the thylakoid membranes. The absolute spectra were recorded in the dark for both polarizations (vertical and horizontal) of the measuring beam, before and after illumination of the sample at 77 K. All four spectra (V and H before and after illumination) were stored in a signal averager (Intertechnique Didac) and could be manipulated so as to obtain light minus dark or linear dichroism spectra.

Horse heart cytochrome *c* was purchased from Sigma Chemical Co., St. Louis, MO. Cytochrome c_2 from *Rps. sphaeroides* was prepared as described by Bartsch [13]. One volume of cytochrome dissolved in water, one volume of Tris · HCl (0.1 M, pH 7.5), and six volumes of 10% (w/v) poly(vinyl alcohol) (Sigma Type III) were mixed and allowed to dry. Sodium ascorbate (10 mM) or potassium ferricyanide (10 mM) had been added to bring about complete reduction or oxidation of the cytochrome. The dried films were stretched as much as 3-fold, and their linear dichroism spectra were measured with an apparatus described previously [14]. A helium flow cryostat (Meric, France) was used for measurements at low temperature.

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*This estimate was made by measuring the absorbance of a film with the measuring beam axis making an angle of either 0° or 60° with the normal to the slide, and with the electric vector in the plane of the slide in both measurements.

Results

A. Orientation of the cytochrome b-559 α-band transition(s) with respect to the membrane plane

It has been shown in many laboratories [15—18] that photooxidation of cytochrome *b*-559 is mediated by the Photosystem II reaction center at temperatures below —100°C, down to liquid helium temperature. We have determined the orientation of the cytochrome *b*-559 α band with respect to the membrane plane by studying the dichroism of the light induced changes related to its photooxidation at 77 K in a suspension of oriented chloroplasts. Absorption spectra of the suspension of oriented chloroplasts were recorded and digitized between 535 and 565 nm at 77 K for vertical and horizontal polarizations of the measuring beam both before and after illumination at that temperature. Such spectra are depicted in Fig. 1, where A_{HD}, A_{HL}, A_{VD} and A_{VL} refer to absolute spectra measured with horizontal or vertical polarization of the measuring beam both before (D) and after (L) illumination. As a check for trivial artifacts, depolarization of the measuring beam, or baseline distortions, one can compute from these absolute spectra the light minus dark difference spectrum one should obtain with an unoriented sample and unpolarized measuring beam, by using the equation [9]:

$$3(A_L - A_D) = (2A_{VL} + A_{HL}) - (2A_{VD} + A_{HD})$$

where the quantity $(A_L - A_D)$ represents the difference between spectra recorded after illumination (A_L) or before illumination (A_D) for a sample of unoriented chloroplasts. A light minus dark difference spectrum calculated in this way from the absolute spectra of Fig. 1 is shown in Fig. 2a. It is indeed identical to those reported for unoriented samples of chloroplasts [15], with the characteristic features of the oxidation of cytochrome *b*-559 (bleaching

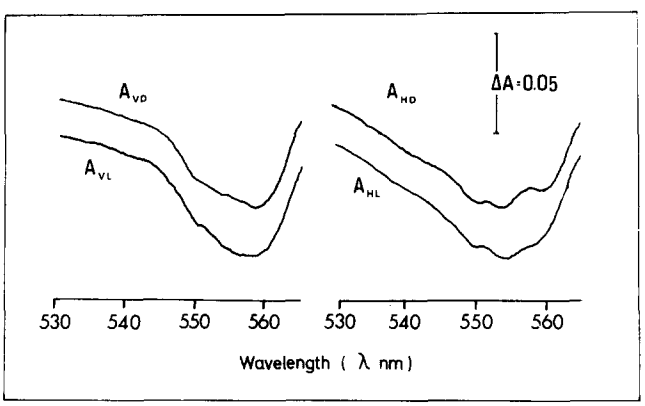

Fig. 1. Absorption spectra of an oriented sample of spinach chloroplasts suspended in 66% glycerol; 33% 0.4 M sucrose, 10 mM Tris · HCl, pH 7.8 (1 mg/ml chlorophyll; optical path 1 cm). A_{VD} and A_{HD} refer to absorption spectra measured before illumination at 77 K with the measuring beam polarized vertically or horizontally, respectively. A_{VL} and A_{HL}, spectra recorded after illumination at 77 K, for vertical or horizontal polarizations of the measuring beam. For vertically or horizontally polarized light the electric vector of the measuring beam was parallel or perpendicular to the membrane plane, respectively.

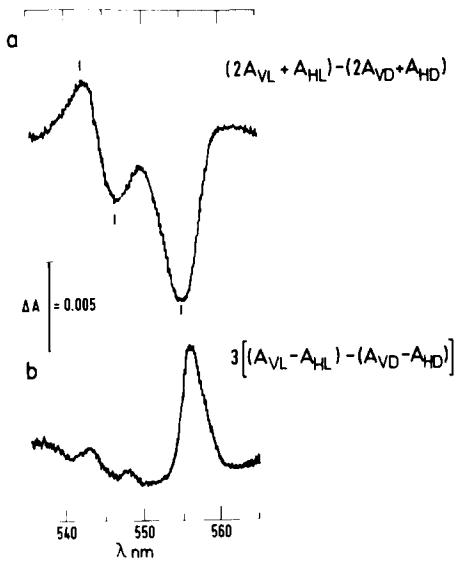

Fig. 2. (a) Light minus dark difference spectrum (unpolarized) calculated from the spectra of Fig. 1 using the equation $3(A_L - A_D) = (2A_{VL} + A_{HL}) - (2A_{VD} + A_{HD})$; see the text. (b) Linear dichroism spectrum in the region of the α band of cytochrome *b*-559 calculated from the spectra of Fig. 1, using the equation $3(A_{VL} - A_{HL}) - 3(A_{VD} - A_{HD})$.

centered at 556 nm) and of the reduction of C550 (S-shaped signal negative at 547 nm and positive at 542 nm). The linear dichroism spectrum (light minus dark; Fig. 2b) is equal to the quantitiy $(A_{VL} - A_{HL}) - (A_{VD} - A_{HD})$. This linear dichroism spectrum shows, first of all, that the signals linked to C550 reduction are not strongly polarized as there are no significant bands in the 535—550 nm region. On the other hand, in the region of the cytochrome *b*-559 α band, around 556 nm, a large signal is observed. The dichroic ratio $(A_{VL} - A_{VD})/(A_{HL} - A_{HD})$ for the α band of the cytochrome *b*-559 was found to lie between 0.3 and 0.4 for five individual measurements, showing that this transition is preferentially oriented perpendicular to the membrane plane. An interesting observation is that the shape of the α band in the linear dichroism spectrum is not symmetrical and the peak is at a greater wavelength, when compared with the unpolarized light minus dark difference spectrum (see Fig. 2). This point will be considered in more detail in the Discussion.

B. Orientation of cytochrome c-558 with respect to the membrane plane of chromatophores of Rhodopseudomonas viridis

We have studied the orientations of the α, β and γ transitions of high potential cytochrome *c*-558 with respect to the membrane plane in chromatophores and whole cells of *Rps. viridis*. Chomatophores were oriented by air drying on glass slides [9]. In order to keep the cytochrome *c*-558 reduced in the dark, 1 mM diaminodurene was added to the suspension of chromatophores before drying. Under these conditions low potential cytochrome *c*-552 was mainly in its oxidized form and therefore made little contribution to the absolute absorbance or light induced absorbance changes in the region of the α and β bands.

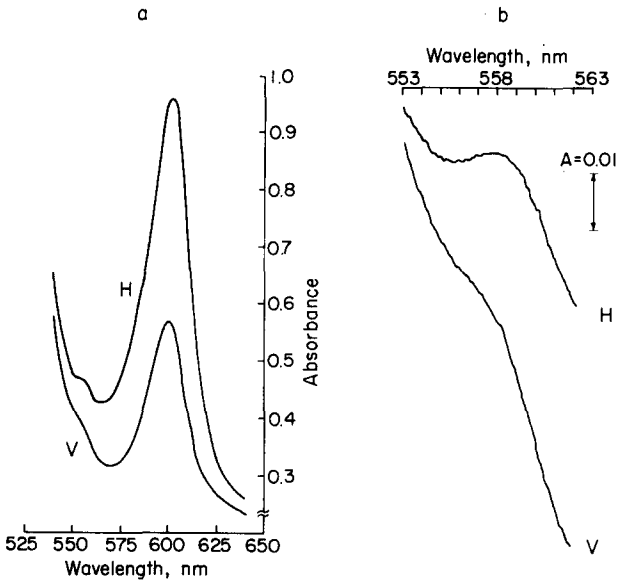

Fig. 3. Polarized absorption spectra of dried chromatophores of *Rps. viridis*, treated with 1 mM diaminodurene to reduce cytochrome *c*-558. The chromatophores were suspended in 5—10 mM Tris · HCl, pH 7.5, and allowed to dry on a glass slide. Absorbance of the dried film was 0.4 at 830 nm. Absorbance was recorded by a Cary 14R Spectrophotometer, with the measuring beam axis making an angle of 60° with the normal to the slide. Glan-thompson polarizers were placed in the reference and sample beams. H and V refer to absorption spectra recorded with measuring light polarized either horizontally or vertically. For vertically polarized light the electric vector was parallel to the plane of the slide. (a) H and V spectra in the range 540—640 nm. (b) The small peak at 558 nm in (a) attributed to reduced cytochrome *c*-558, is magnified 10-fold.

Whole cells were oriented by applying a magnetic field (20 kgauss) at room temperature [10].

The linear dichroism spectrum of chromatophores oriented by air drying is shown in Fig. 3. It is clear that the α band of reduced cytochrome *c*-558 absorbs more with horizontally polarized light (H) than with vertically polarized light (V). However, the strong absorption by antenna pigments (especially carotenoids) makes it difficult to determine from these spectra the exact orientation of the cytochrome *c*-558 α band transition with respect to the membrane plane. One way to circumvent this problem is to examine the light-induced changes related to cytochrome *c*-558 photooxidation, using actinic flashes. Fig. 4 shows the kinetics of light-induced changes of cytochrome *c*-558 photooxidation in dried chromatophores, using polarized light at a few selected wavelengths. The complete point by point oxidized minus reduced spectrum in the regions of the α and β bands is shown in Fig. 5. It is apparent that around the α band the 'oxidized minus reduced' negative peak is more intense when measured with horizontally polarized light. Around the β band there is no significant difference between the intensity of V and H spectra at their extrema, but the peak wavelengths differ by about 2 nm. The dichroic ratio is not constant throughout either the α band (negative peak at 558 nm) or the β band (negative peak near 525 nm); this is most clear in the fact that the two polarizations yielded different isosbestic points (Fig. 5). Turning to whole cells of *Rps.*

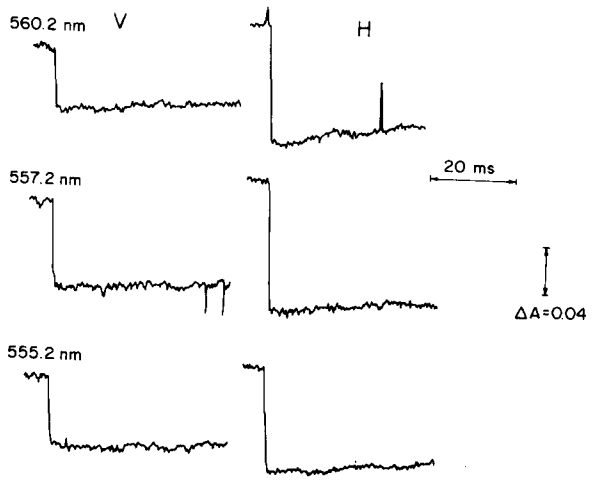

Fig. 4. Light-induced absorbance changes at the indicated wavelengths in chromatophores of *Rps. viridis*, dried on glass slides as described for Fig. 3. Absorbance of the dried film was 2.5 at 830 nm. The slide was placed in relation to the measuring beam as in Fig. 3, with horizontal (H) and vertical (V) polarization provided by a Glan-Thompson polarizer. Actinic light was provided by a xenon flash; see Materials and Methods. The actinic flash was unpolarized and was saturating, so as to avoid artifacts due to photoselection. Photooxidized cytochrome *c*-558 reverted slowly to the reduced form in the dark; this could be accelerated by dipping the slide briefly in a solution of diaminodurene and allowing it to dry again.

viridis oriented magnetically, Fig. 6b shows the absorbance changes occurring at 430 nm induced by a series of four saturating flashes for both polarizations H and V of the measuring beam. The redox potential of the suspension was

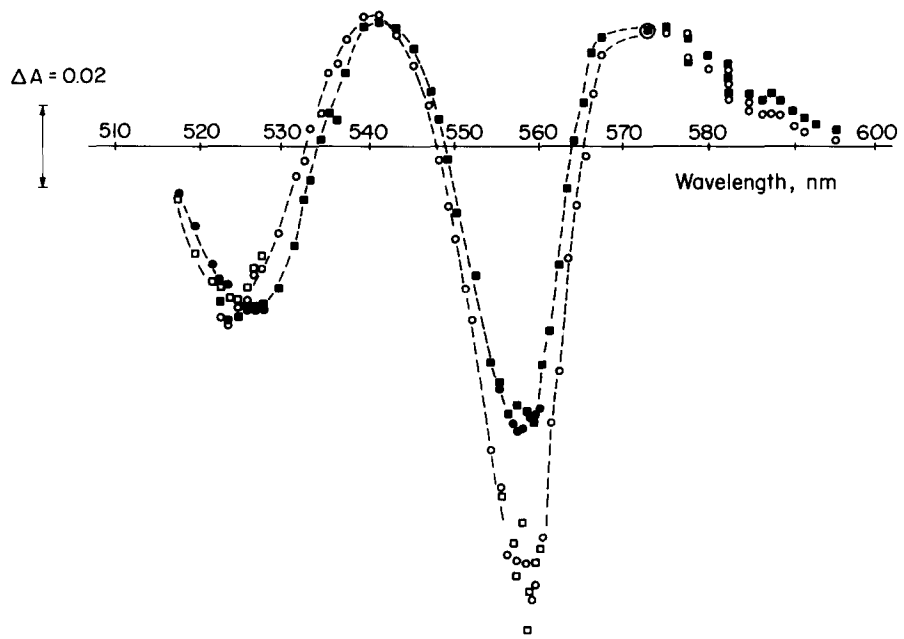

Fig. 5. Point-by-point spectrum of light-induced absorbance changes in dried chromatophores of *Rps. viridis*. The points in this plot were from measurements as illustrated in Fig. 4. Open symbols indicate horizontally polarized measurements; full symbols, vertically polarized measurements.

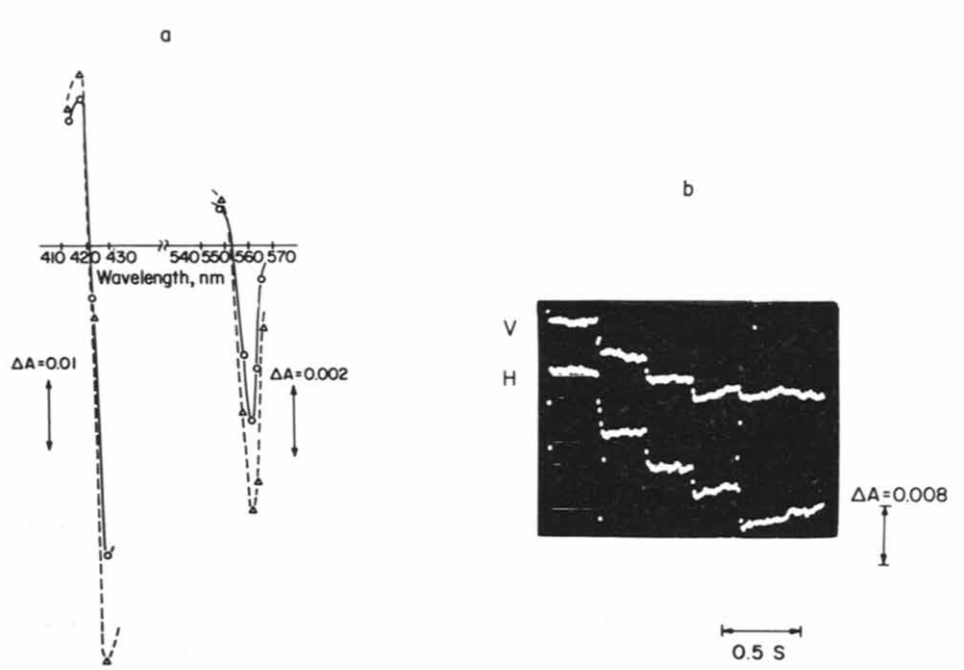

Fig. 6. Light-induced absorbance changes in *Rps. viridis* cells oriented in a magnetic field. The cells were suspended in 33% glycerol, 6.6 M Tris · HCl, pH 7.8, to an absorbance of 0.75 at 1010 nm. The applied magnetic field was of 20—24 kG. Dye-laser flashes, 600 nm, were applied from the top of the cuvette as single flashes or as a train of flashes 400 ms apart. A Corning 4-96 (blue) filter was placed in front of the measuring beam, which was polarized either horizontally (H) or vertically (V) through a Rochon polarizer. The applied magnetic field was parallel to the electric vector with horizontal polarization. (a) Oxidized-minus-reduced spectra obtained by horizontally (△- - - - -△) and vertically (○———○) polarized measurements. (b) An illustration of absorbance changes at 430 nm, in response to a train of flashes. The response to the first flash in each series was taken for the point-by-point spectra in trace (a) on the left.

+200 mv and under such experimental conditions we observed only light-induced changes due to cytochrome *c*-558 photooxidation [2]. In Fig. 6a the difference spectra of the α and γ bands are plotted from experiments similar to the one reported in Fig. 6b. Only the absorbance change occurring on the first flash was used in constructing these spectra. One can note again, as in Fig. 5, the large difference in amplitude for absorbance changes recorded with vertical and horizontal polarization of the analyzing beam ($\Delta A_V/\Delta A_H = 0.6$ for the α band), and that the dichroic ratio is not constant throughout the α band.

Discussion

Two main observations are reported in this study of the polarization of light induced changes linked to photooxidation of cytochromes in photosynthetic membranes. High orientations are observed ($\Delta A_V/\Delta A_H \leqslant 0.6$) for both high potential cytochrome *b*-559 (spinach) and high potential cytochrome *c*-558 (*Rps. viridis*) in their α bands, but these dichroic ratios are not constant through the α or β bands. This is seen clearly in Fig. 2, where the linear dichroism spectrum of the cytochrome *b*-559 α band is not symmetrical and

where the maximum of the band is shifted by a few nanometers compared to the maximum observed in the unpolarized light minus dark absorption spectrum. Also the bleaching of the β band of cytochrome *c*-558 occurs at slightly different wavelengths in the spectra recorded with horizontal or vertical polarization of the measuring beam (see Fig. 5).

The changes of the dichroic ratio throughout both α and β bands for cytochrome *b*-559 and cytochrome *c*-558 can be explained by supposing that each band is composed of a minimum of two transitions oriented differently and absorbing at slightly different wavelenths. The occurrence of two transitions absorbing at different wavelengths, for both α and β bands, can arise in at least two distinct ways: (1) We know that for both cytochrome *b*-559 of spinach chloroplasts and cytochrome *c*-558 of *Rps. viridis*, two functionally equivalent molecules are bound to their respective reaction centers [19—21] and we can therefore speculate that these two cytochrome molecules absorb at slightly different wavelengths in both the α and β band regions. (2) The α and β bands are each split into two or more distinct transitions within the cytochrome molecule itself.

In order to evaluate these possibilities we have sought evidence for complexity in the α and β bands of other cytochromes. According to molecular orbital theory for simple porphyrins [22] the intense absorption band near 400 nm (γ or Soret band) and the less intense bands in the 500—600 nm region (α and β bands) result from $\pi \rightarrow \pi^*$ transitions. These transitions (α, β and γ) are predicted to be isotropically polarized within the heme plane (i.e., x and y axes are approximately equivalent) because of the 4-fold symmetry of metalloporphyrins [22]. However, inbalance of x and y transitions can arise from asymmetric potential fields due to axial ligands, side chains and electrostatic effects of binding proteins [23]. For example, polarized absorption studies of single crystals of oxidized horse heart cytochrome *c* [24] show that the γ and β transitions are indeed isotropically polarized, whereas the α band exhibits some deviation, suggesting that x and y transitions are not equivalent: in the α band, one polarization is 20% stronger than the other [24].

The presence of two or more distinct transitions in the α bands of reduced *c*-type cytochromes is revealed in absorption spectra at low temperature [25], and by the magnetic circular dichroism technique [26]. As an example, Fig. 7a shows the absorption spectrum of the cytochrome *f* of barley, recorded at 77 K in the α band region. Two transitions, at 552 and 548 nm, are evident. Although such splitting is not observed in the case of cytochrome *b*-559 (Fig. 7b), comparison of the α band widths of cytochromes *f* and *b*-559 (Fig. 7) suggests that the α band of cytochrome *b*-559 is composed of two transitions occurring at slightly different wavelengths. Further evidence that x- and y-polarized transitions occur at slightly different wavelengths in both the α and β bands comes from the linear dichroism spectra of *c*-type cytochromes oriented in poly(vinyl alcohol) films. Fig. 8 shows the absorption (a) and linear dichroism (b) spectra of reduced (full line) and oxidized (dotted line) horse heart cytochrome *c* in stretched poly(vinyl alcohol) films. Strong variations of the linear dichroism occur within both the α and β bands, especially in the reduced film. The resolution of differently polarized transitions is more dramatic at 77 K (Fig. 8c and d). Similar absorption and linear dichroism spectra

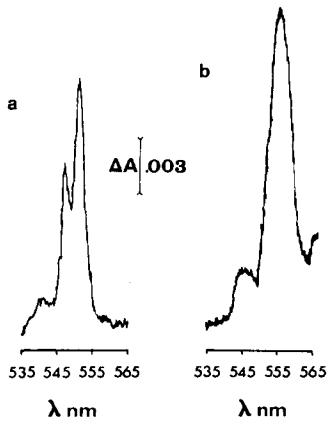

Fig. 7. (a) Absorption difference spectrum of reduced minus oxidized cytochrome *f* of barley etioplasts (lacking cytochrome *b*-559) recorded at 77 K with a Perkin-Elmer 356 double beam spectrophotometer. The spectrum was obtained by measuring the difference in absorption between a suspension reduced with 10 mM ascorbate and one oxidized with 10 mM ferricyanide. (b) Absorption difference spectrum of reduced minus oxidized cytochrome *b*-559 (high potential) in barley chloroplasts recorded at 77 K. This spectrum was also obtained by measuring the difference in absorption between a suspension reduced with ascorbate and one oxidized with ferricyanide. The reduced sample was illuminated at room temperature by far-red light before freezing, in order to photooxidize the cytochrome *f*.

are observed in the case of cytochrome c_2 from *Rps. sphaeroides* (data not shown). Unfortunately, purified cytochrome *b*-559 and cytochrome *c*-558 were not available to us to test the splitting of their α and β bands in stretched poly-(vinyl alcohol) films. These linear dichroism spectra of *c*-type cytochromes oriented in stretched poly(vinyl alcohol) films show definitively the intramolecular complexity of the α and β band structures.

In the light of the results obtained on stretched films and low temperature absorption spectra of reduced pure cytochromes, we prefer to interpret the inhomogeneity in the linear dichroism spectra of the α and β bands of cytochrome *b*-559 and cytochrome *c*-558 in vivo by assuming that *x* and *y* transitions occur at slightly different wavelengths for each cytochrome molecule. However, we cannot completely rule out the possibility that each of the two cytochrome molecules bound to the reaction center [19,21] has a different orientation with respect to the membrane plane, and slightly different absorption maxima for both α and β bands.

We can rule out the possibility that anomalous dispersion (change of refractive index through an absorption band) contributed significantly to these complex dichroic phenomena, on two grounds. First, the variations of linear dichroism occurred over wavelength intervals considerably less than the absorption bandwidths. Second, no such variations were seen in the spectra of bacteriochlorophyll and carotenoid pigments.

It is difficult to calculate an accurate angle between the cytochrome heme plane and the membrane plane because of the uncertainties of *x* and *y* attribution in the absorption spectra and because the degree of orientation of the membrane is not known precisely. However, we can estimate that the angle between the heme plane and the membrane plane is greater than 35° for both high potential cytochrome *c*-558 and *b*-559. For *Rps. viridis* the dichroic ratio

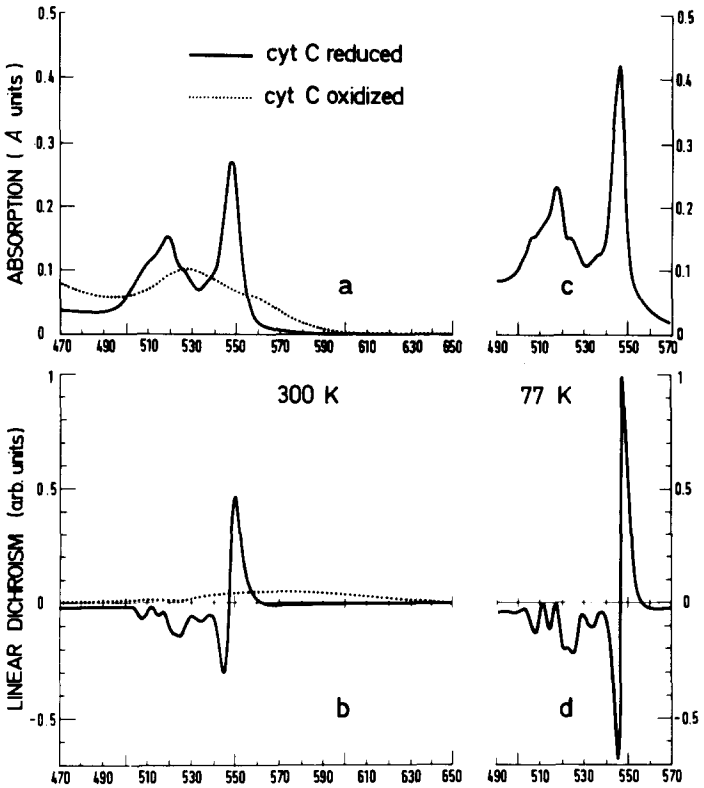

Fig. 8. (a) Absorption spectra of reduced (———) and oxidized (. . . . . .) horse heart cytochrome *c* embedded in poly(vinyl alcohol) films, recorded with a Cary 17D Spectrophotometer at room temperature. (b) Linear dichroism spectra ($A_{\parallel} - A_{\perp}$) of stretched poly(vinyl alcohol) film of reduced (———) and oxidized (. . . . . .) horse heart cytochrome *c*, $A_{\parallel}$ and $A_{\perp}$ refer to absorption parallel and perpendicular to the stretch axis, respectively. (c) Absorption spectra of reduced cytochrome *c* recorded at 77 K. (d) Linear dichroism spectra ($A_{\parallel} - A_{\perp}$) recorded at 77 K of a stretched film of reduced cytochrome.

$\Delta A_V/\Delta A_H$ of cytochrome *c*-558 is close to 1.0 in the β band and 0.6 in the α band, for chromatophores measured as in Figs. 4 and 5. The spectra of Fig. 5 are consistent with the following: The β band contains x- and y-polarized transitions of equal intensity ('planar transition moment') but slightly different energies (different peak wavelengths), with the average heme plane making an angle of 55° with the membrane plane. In the α band the x- and y-polarized transitions are of the same energy but of unequal intensities, so that their sum has a strong linear component (a high degree of ellipticity).

Our optical studies of cytochrome orientation are consistent with the EPR work of Prince et al. [6], who reported that the heme plane of high potential cytochrome *c*-558 lies nearly perpendicular to the membrane plane in *Rps. viridis*. The same authors also reported [5] that the high potential cytochrome *c*-555 in *C. vinosum* is perpendicular to the membrane plane. Perhaps all of the high potential cytochromes in photosynthetic tissues lie preferentially perpendicular to the membrane plane. It should be noticed that the heme planes of several cytochromes have been found to be oriented rather perpendicular to the plane of membranes from non-photosynthetic tissues. In mitochondria, the

hemes of the cytochrome oxidase (both a and a_3), and of cytochromes c and b are oriented perpendicularly to the membrane [27]. A similar orientation has been observed for cytochrome d in an oriented membrane fraction from *Tetrahymena pyriformis* [28]. In contrast, an orientation rather parallel to the membrane plane has been detected for the hemes of cytochrome P-450 in microsomes [29] and of the low potential cytochrome c-553 in *C. vinosum* [7]. These observations suggest that orientation of the cytochrome molecules in biological membranes might have some role in the electron transfer process.

EPR spectroscopy on oriented membranes has definite advantages compared to polarized absorption spectroscopy in analyzing the orientations of cytochrome molecules. This is due to the fact that the g values for the x and y transitions are different and that a third axis (z), perpendicular to the heme plane, can be monitored. However, the results presented here show that the α and β bands are not always degenerate and that the orientation of the x- and y-polarized transitions can be monitored separately. This should allow us to unify the assignment of the x and y axes as seen by EPR with the x- and y-polarized transitions seen by polarized absorption. Furthermore, EPR can monitor only the oxidized species, while polarized light spectroscopy is most useful with the reduced species. By using these two techniques on the same biological material it might be possible to detect changes in orientation occurring upon modification of the redox state of the cytochrome.

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