Biochimica et Biophysica Acta, 462 (1977) 706–717

BBA 47407

THE LIGHT-INDUCED CAROTENOID ABSORBANCE CHANGES IN *RHODOPSEUDOMONAS SPHAEROIDES*

AN ANALYSIS AND INTERPRETATION OF THE BAND SHIFTS

MARC SYMONS, CHRISTINE SWYSEN AND CHRISTIAAN SYBESMA

Biophysics Laboratory, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels (Belgium)

(Received May 4th, 1977)

SUMMARY

An analysis has been made of the spectrum of the carotenoid absorption band shift generated by continuous illumination of chromatophores of the G1C-mutant of *Rhodopseudomonas sphaeroides* at room temperature by means of three computer programs. There appears to be at least two pools of the same carotenoid, only one of which, comprising about 20 % of the total carotenoid content, is responsible for the light-induced absorbance changes. The 'remaining' pool absorbs at wavelengths which were about 5 nm lower than those at which the 'changing' pool absorbs. This difference in absorption wavelength could indicate that the two pools are influenced differently by permanent local electric fields.

The electrochromic origin of the absorbance changes has been demonstrated directly; the isosbestic points of the absorption difference spectrum move to shorter wavelengths upon lowering of the light-induced electric field. Band shifts up to 1.7 nm were observed. A comparison of the light-induced absorbance changes with a KCl-valinomycin-induced diffusion potential has been used to calibrate the electrochromic shifts. The calibration value appeared to be 137 ± 6 mV per nm shift.

INTRODUCTION

Light-induced changes of the absorption in the 400–600 nm spectral region in cells and cell-free preparations of higher plants, algae and photosynthetic bacteria have been studied extensively [1–21]. There is a general agreement that at least a large part of these changes in absorption reflect the generation of an electrical potential across the thylakoid or chromatophore membrane. A strong indication of this is the similarity between the light-minus-dark difference spectrum observed in chloroplasts and the difference spectra obtained by subjecting a layer of pigments (a mixture of carotenoids and chlorophyll) to an electrical field [4–7]. Similar experiments have been carried out with dry films of chromatophores of *Rhodospirillum rubrum*, showing a good agreement between absorption changes induced by light and by an external field [8]. In chromatophores, applied diffusion potentials cause absorbance changes similar

to those induced by light [9]. These results, as well as the effect of phosphorylating conditions and ionophores on the decay of the absorbance change after a single flash [11–13] also substantiate the hypothesis of an electrical origin of the absorbance change.

However, there is still no unambiguous experimental evidence to support a specific electrochromic mechanism that quantitatively would explain the spectral phenomena. The original interpretation of light-induced (and diffusion potential-induced) absorbance changes in chromatophores from *Rhodopseudomonas sphaeroides* suggested a red shift of approximately 10 nm in, at the most, 10 % of the carotenoid molecules [14–17]. Such a shift, however, seems to be too large to be caused by an electrochromic effect. Moreover, contrary to expectations, no appreciable shift of the isosbestic point in the difference spectra has been observed. Amesz et al. [14] assumed that the extent of the light-induced shift does not change with light intensity, but that the amount of pigment undergoing the shift does. Conjeaud and Michel-Villaz [18], on the other hand, proposed a hypothesis in which the larger part of the shift originates from an electrochromic change in the extinction coefficient.

Recently, de Grooth and Amesz [22] obtained experimental results suggesting the existence of two spectrally different pools of the same carotenoid, only one of which exhibited electrical field-induced changes of absorbance. The results described in the present paper confirm qualitatively such a hypothesis but show significant quantitative differences.

MATERIALS AND METHODS

Chromatophore preparation

Rps. sphaeroides wild type and the mutants G1C and R-26 (obtained by courtesy of Dr. A. Crofts, University of Bristol, U.K.), were grown anaerobically in a medium as described by Cohen-Bazire et al. [23]. After harvesting from 2-day old cultures, the cells were washed first in distilled water and then in 100 mM choline chloride, pH 7.8. The whole cells were resuspended in a medium containing 200 mM glycyl glycine and 100 mM choline chloride, pH 7.8, and disrupted by sonic treatment during two 1-min periods (except where stated), with an interval of 4 min, in an MSE 150 W Disintegrator at maximum power; the suspension was kept cool during sonication by an ice jacket. The fraction of this suspension sedimenting between 20 000 $\times g$ (30 min) and 110 000 $\times g$ (90 min) was collected and washed in a medium containing 10 mM glycyl glycine and 10 mM choline chloride, pH 7.8. The chromatophore fraction was resuspended in a minimal volume of the washing medium and stored under nitrogen at low pressure at 4 °C.

Spectrophotometric methods

Measurements were carried out with a Cary-17 spectrophotometer, maximum sensitivity 0.1 *A* full scale, provided with an actinic light source. The wavelength of the actinic light was selected by a 861 nm Balzer interference filter. Unless stated otherwise, the intensity of the actinic light was chosen such that responses of about 75 % of the saturation value were obtained, in order to remain in the linear part of the light saturation curve. The optical pathlength was 1 cm. The spectral bandwidth varied from 0.5 nm (in the 500 nm region) to almost 1 nm (at 410 nm, where noise problems were the limiting factor). Experiments were carried out at room temperature.

Absorbance changes induced by a diffusion potential of KCl in the presence of valinomycin, were measured with a double beam spectrophotometer constructed in the laboratory. The light outputs of two Bausch and Lomb 500 mm^2 reflection grating monochromators were alternatively chopped at 143 cps by a rotating disk. The mixed beam was passed through a sample vessel (1 cm pathlength) by a lightpipe-system (custom-made by Schott-Jena-Glasswerk) and measured with an EMI 9524 B photomultiplier. A Brookdeal Lock-in-Amplifier, type 401 A was used to detect the signals. The two wavelengths were chosen such that absorbance of the sample at each wavelength was equal, thus avoiding the necessity for correction for the dilution induced changes by adding a KCl or valinomycin solution to the sample.

Computer programs

In order to analyze the measured spectra three computer programs were designed. The first one, labeled DIFSPC, was used to establish the absorption spectrum of the pigments responsible for the light-induced absorption changes. The program compares each of the three parts of the difference spectrum with the difference of two Gaussian curves, allowing for changes in peak height (extinction changes) in one of them. It consists mainly of a minimization routine, called MINUIT (a subroutine of the CERN Computer Center Program Library, kindly made available to us by the Interuniversitary Research Center for Elementary Particles). The function to be minimized is the following:

$$\left[\Delta(\lambda)+\sum_{i=1}^{3}\alpha_i \exp\left\{-\left(\frac{\lambda-\lambda_i}{w_i}\right)^2\right\}-\sum_{i=1}^{3}\alpha_i(1+\Delta\varepsilon_i)\exp\left\{-\left(\frac{\lambda-\lambda_i-\Delta\lambda_i}{w_i}\right)^2\right\}\right]^2$$

in which $\Delta(\lambda)$ is the measured difference spectrum. Five parameters were used to determine each of the three shifting Gaussian curves. These are the fraction of the total absorption, α_i, which is involved in the bandshift, the wavelength of the peak centre λ_i, the e^{-1}-half bandwidth w_i, the light-induced band shift $\Delta\lambda_i$, and the light-induced fractional change of the extinction coefficient $\Delta\varepsilon_i$.

The second program, labeled FITCAR, was used to compare the spectra obtained by DIFSPC with the absorption spectrum of the chromatophore suspension. It also uses the MINUIT minimization subroutine.

The third program, labeled CORRELA, was used to obtain an independent confirmation of the DIFSPC results. In this program two kinds of correlation functions were used: $C_{LD}(\lambda)$ is the cross-correlation between the absorption spectrum in the light and in the dark. In order to obtain the information from this function, the auto correlation-function $C_{DD}(\lambda)$ of the dark spectrum was subtracted. In a simplified version, the absorption spectrum in the dark, $D(\lambda)$, can be considered as comprising two Gaussian curves in proportions X and $1-X$, each centered at the same wavelength λ_o. One of these (proportion X) shifts in the light over $\Delta\lambda$, the other doesn't. Then

$$C_{LD}(\lambda)-C_{DD}(\lambda)=\sum_{i=410}^{530}\left[\exp\left\{-\left(\frac{\lambda_i-\lambda_o-\Delta\lambda}{w}\right)^2\right\}X+\exp\left\{-\left(\frac{\lambda_i-\lambda_o}{w}\right)^2\right\}(1-X)\right]$$

$$\times\exp\left\{-\left(\frac{\lambda_i-\lambda_o+\lambda}{w}\right)^2\right\}-\sum_{i=410}^{530}\exp\left\{-\left(\frac{\lambda_i-\lambda_o}{w}\right)^2\right\}\exp\left\{-\left(\frac{\lambda_i-\lambda_o+\lambda}{w}\right)^2\right\}$$

From this equation it can be calculated that the wavelength at which the difference between the two correlation functions is zero (the isosbestic point) is equal to $\Delta\lambda/2$, which is also true for the isosbestic point of the difference spectrum $L(\lambda)-D(\lambda)$, in which $L(\lambda)$ is the absorption spectrum in the light. Furthermore, $C_{LD}(0)-C_{DD}(0)$ appears to be a linear function of the shifting fraction X, and an increasing function of the shift $\Delta\lambda$. However, the actual case is far more complicated. Therefore, in order to obtain a supplementary check, we compared (again by means of the subroutine MINUIT) the experimental correlation difference with a correlation difference computed with values of the parameters obtained by DIFSPC.

All calculations were carried out using units of wavenumbers, rather than wavelengths.

RESULTS AND INTERPRETATION

The computer programs described in the previous section were used to analyze light-minus-dark difference spectra in the spectral region 410 to 530 nm obtained from point by point measurements using *Rps. sphaeroides* mutant G1C chromatophores. Such a difference spectrum is shown by the dots in Fig. 1. The spectrum was corrected for light-induced absorbance changes due to the reactions of photosynthetic electron transport components. The correction was carried out by subtracting the residual light-minus-dark absorption difference spectrum obtained after addition of 50 μM gramicidin. At this concentration of gramicidin the carotenoid absorbance changes seemed to be eliminated. At 503 nm the correction involved about 5 % of the difference spectrum at that wavelength. Also, within experimental error, at 430 nm the difference spectrum in the presence of gramicidin is identical to the difference spectrum

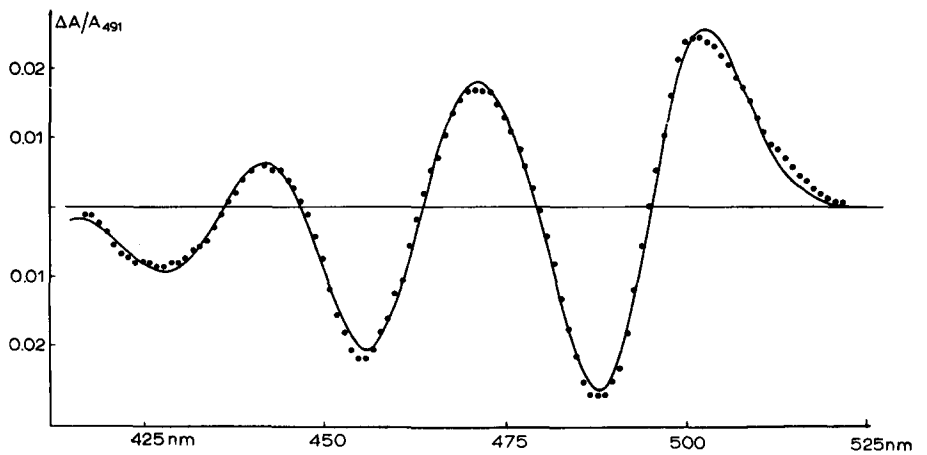

Fig. 1. Light-induced and computer-fitted carotenoid absorption difference spectra of chromatophores from the G1C mutant of *Rps. sphaeroides*. Chromatophores at a concentration of 21 μM (determined by using the extinction coefficient of bacteriochlorophyll in vivo given by Clayton [31]) were suspended in 10 mM glycylglycine and 10 mM choline chloride, pH 7.8. Circles, experimental absorption difference spectrum obtained by point by point measurements and corrected for non-carotenoid absorbance changes (see text); solid line, fit by the DIFSPC computer program, giving the parameters compiled in Table I.

in the absence of gramicidin. This would suggest that within the spectral range considered the correction for non-carotenoid absorbance changes could be carried out by this method.

The drawn line in Fig. 1 is a DIFSPC computer program fit (see Materials and Methods). Best fits with approximately equal least square minima were obtained for a number of combinations of the 3×5 parameters determining the computed difference spectrum. In all such combinations the e^{-1}-bandwidths w_i remain invariant within 1 %. Some of the other parameters have complementary relations in respect to each other. Thus, it appeared that the fit obtained by assuming a large fraction of the total absorption (α_i) shifting over a short wavelength distance ($\Delta\lambda_i$) is as good as a fit obtained by assuming a smaller fraction shifting over a larger wavelength distance. A similar complementary relation exists between the shifting fraction and the change in extinction coefficient. These relationships can be expressed in the following empirical equations:

$$\alpha_i \times \Delta\lambda_i = K_i (i = 1, 2, 3) \tag{1}$$

$$\alpha_i \times \Delta\varepsilon_i = L_i (i = 1, 2, 3) \tag{2}$$

in which K_i is constant within 1 % and L_i is constant within 20 %. The constant K_i, which for the three bands has ratios $K_1 : K_2 : K_3 = 1 : 0.80 : 0.56$, is proportional to the maximum absorbance change (which appears to be located at 503 nm). This has been verified by measurement of the difference spectrum at a lower intensity of the actinic light. Thus, we can write: $K_i = C_i \Delta A_{max}$, in which C_i is a constant and ΔA_{max} is the maximum absorbance change (at 503 nm). Furthermore, as Conjeaud et al. [18] have pointed out, an electrochromic change of the extinction coefficient ($\Delta\varepsilon_i$) influences the location of the isosbestic point λ_i^I. Using realistic estimates for the band parameters, this influence was calculated for different values of the extinction coefficient change. The result of this calculation can be expressed in the following equation:

$$\Delta\lambda_i^I / \Delta\varepsilon_i = 0.25 \tag{3}$$

in which $\Delta\lambda_i^I$ is the change in location of the isosbestic point. According to Eqn. 3 a change in extinction of 1 % would result in a displacement of the isosbestic point by 0.25 nm.

From Eqns. 1, 2 and 3 it can be deduced that, if it can be assumed that the fraction of the total absorption that shifts (α_i) is equal for each of the three peaks comprising the computed spectrum, only one parameter has to be known in order to determine the others and, thus, select a 'true' best fit. The assumption seems to be a reasonable one, since the three bands of the total carotenoid absorption spectrum originate from one molecule. According to Labhart [24] the three bands can be assumed to result from a vibrational splitting of one electronic transition. Thus, one should expect that all three bands are influenced to the same extent by an external electrical field. Moreover, preliminary experiments on solvatochromic effects on carotenoids (β-carotene) have shown that the three absorption bands shift as a whole: the ratios between the peak heights remained constant within 2 % when shifts as much as 10 nm were evoked (Symons, M. et al., unpublished). In such experiments it also appeared that distances between peak wavelengths of the three bands increase when the spectrum shifts to the red. This agrees qualitatively with the empirically found

ratios between the K_i in Eqn. 1. Solvatochromic effects can be seen as analogous to electrochromism since solvent molecules are able to create a 'reaction field' causing Stark effect-like phenomena in pigment molecules [25].

The parameter most amenable to experimental determination appeared to be the shift $\Delta\lambda_1$, of the red-most peak. In order to find an experimental value for this parameter we followed the location of the isosbestic point at different actinic light intensities. Plots were made of the wavelength of the isosbestic point versus the maximum absorbance change (cf. Fig. 6). It can be shown that for shifts not exceeding 5 nm this relation should be linear. The wavelengths of the isosbestic point were then extrapolated to zero absorbance change. From the work of Conjeaud et al. [18] it can be concluded that an (electrochromic) change of the extinction coefficient would only displace the linear plots in a parallel fashion; the wavelength shifts determined from the extrapolations thus do not depend on changes of the extinction coefficient and reflect only (electrochromic) band shifts. Typical values obtained this way were $\Delta\lambda_1 = 1.5 \pm 0.3$ nm (for the long wavelength band) and $\Delta\lambda_2 = 1.0 \pm 0.5$ nm for peak 2. Other estimates of the shifts $\Delta\lambda_1$ and $\Delta\lambda_2$ were obtained by extrapolating to zero intensity of the shift to the blue of the whole difference spectrum which occurs on lowering the actinic light intensity.

Fig. 2 shows the long wavelength part of the difference spectrum at two different actinic light intensities, both corrected for non-carotenoid absorbance changes. Values obtained from these experiments were: $\Delta\lambda_1 = 1.9$ and $\Delta\lambda_2 = 1.3$. The ratio between $\Delta\lambda_1$ and $\Delta\lambda_2$ in both types of experiments agrees within experimental error with the empirically found ratios of the K_i Eqn. 1. The obtained values for $\Delta\lambda_1$ range within boundaries set by the two other computer programmes (see below).

Taking $\Delta\lambda_1 = 1.75$, the 'true' DIFSPC-fit has the parameters given in Table I. The results clearly indicate that the carotenoid molecules responsible for the light-induced absorption change form a pool which is different from the major part of the carotenoid content. Thus, we can conclude that there are at least two populations of

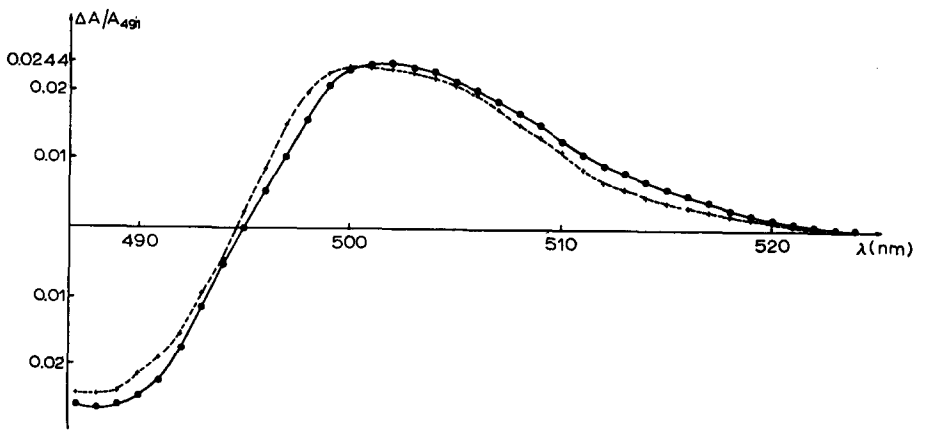

Fig. 2. Long-wavelength part of the light-induced absorption difference spectra of a suspension of *Rps. sphaeroides* G1C chromatophores obtained with actinic light intensities of 11.7 nE/cm² s (dots, solid line) and of 4.5 nE/cm² s (crosses, dashed line). Suspension conditions as in Fig. 1. The lower intensity spectrum was normalized in respect to the higher intensity spectrum.

TABLE I

PARAMETERS OF THE POPULATION UNDERGOING AN ELECTROCHROMIC SHIFT

Band	Centre (nm)	$\frac{1}{2}e^{-1}$-width (nm)	Fraction (% of total absorbance)	Electrochromic extinction change (%)	Shift (nm)
1	494.5	10.5	19	−0.6	1.75
2	463	10.3	19	−1.3	1.4
3	436.5	8.9	19	−0.8	1.0

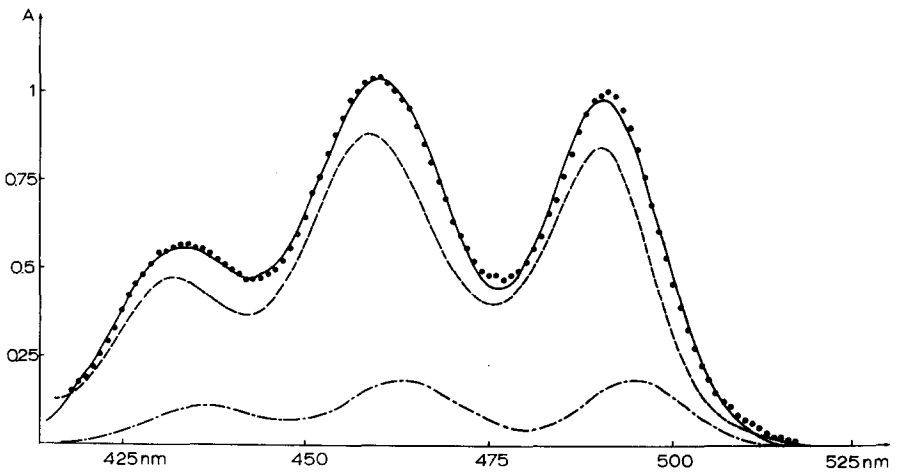

Fig. 3. Analysis of the in vivo carotenoid absorption spectrum. Dashed-dotted line, spectrum of the shifting carotenoid pool (data from Table I); dashed line, spectrum of the remaining carotenoid pool; dots measured in vivo absorption spectrum corrected for non-carotenoid absorption (see text); solid line, FITCAR computer fit of the sum of the shifting spectrum and the remaining spectrum, giving the parameters compiled in Table II.

TABLE II

PARAMETERS OF THE POPULATION REMAINING UNSHIFTED

Band	Centre (nm)	$\frac{1}{2}e^{-1}$-width (nm)	Fraction (% of total absorbance)	Centre of total absorption spectrum (nm)	
				Corrected	Non-corrected
1	489.5	10.9	84	490.5	490.5
2	459.5	12.0	85	459	459.5
3	431.5	9.6	89	431.5	433.5

the same carotenoid, which in the G1C-mutant is predominantly neurosporene (96 %) (see ref. 17).

The absorption spectra of these two moieties were computed by the program FITCAR. This was done by subtracting the sum of the DIFSPC-computed Gaussian curves from an absorption spectrum of a chromatophore suspension from the *Rps. sphaeroides* G1C-mutant. The latter spectrum was corrected for non-carotenoid absorption by using a normalized spectrum of the carotenoidless mutant R-26. The corrected spectrum was very simular to the spectrum of neurosporene in vitro [17]. The spectrum of the non-shifting pool was analyzed by means of three Gaussian curves. Fig. 3 shows the carotenoid spectrum decomposed in a shifting and a non-shifting population. The parameters of the three Gaussian curves of the non-shifting pool are given in Table II.

The table also contains the wavelengths of maximum absorption of the chromatophores, corrected for non-carotenoid absorption together with the non-corrected values. Obviously, there is a discrepancy between the two values: the correction appears to be necessary in order to calculate good estimations for the distance between the two pools.

The computation with the FITCAR program sets an upper boundary on the size of the shifting fraction of 40 %: when this fraction becomes too large, it appeared to be impossible for FITCAR to find a reasonable fit for the spectrum of the remaining part, which became quite distorted. In this way a lower boundary of 0.8 nm is found for the shift, $\Delta\lambda_1$.

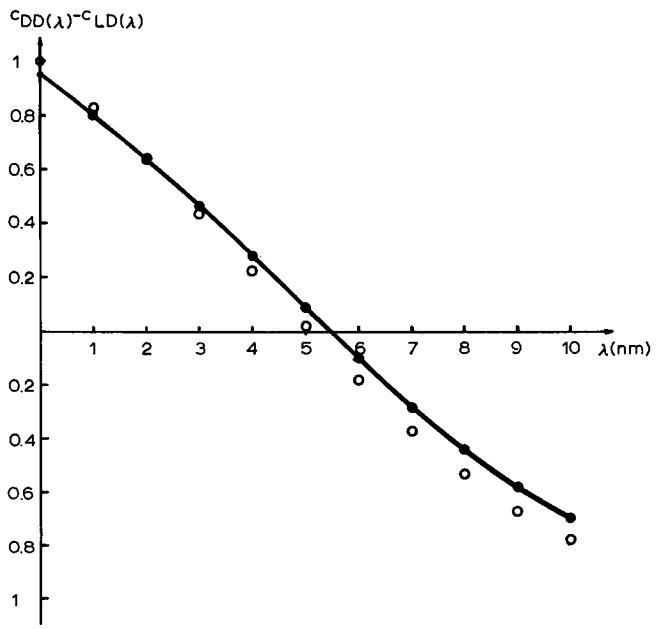

Fig. 4. CORRELA computer fit. Open circles, normalized experimental correlation difference spectrum; solid line and closed circles, CORRELA computer fit giving the parameter values compiled in Table I except those of the extinction coefficient change. The values of the latter parameter in the best CORRELA fit were 40 % lower than those given in Table I.

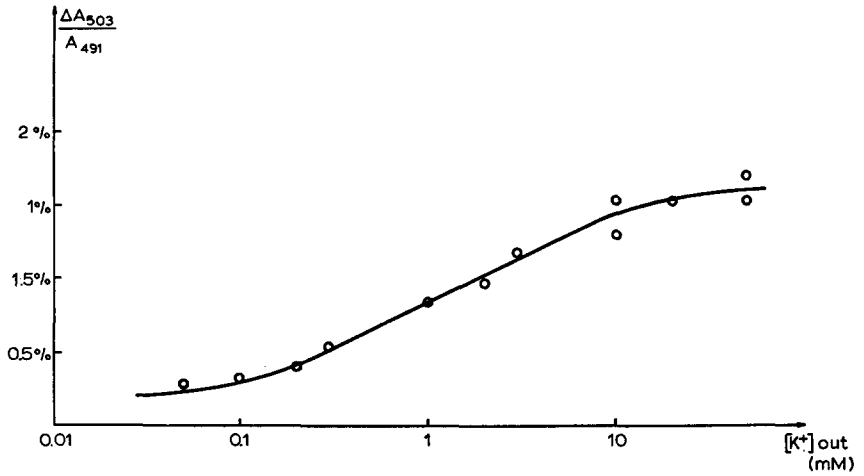

Fig. 5. Absorption difference, $\Delta A = \Delta A_{503}/A_{491}$ as a function of the concentration of a KCl pulse administered in the presence of 1 μM valinomycin to a suspension of *Rps. sphaeroides* G1C chromatophores. The absorption changes were calculated from absorption changes $A_m = A_{488} - A_{470}$ measured with a double beam spectrophotometer. The two wavelengths 488 and 470 nm (of equal absorbance) were choosen to avoid correction for dilution effects (see Materials and Methods).

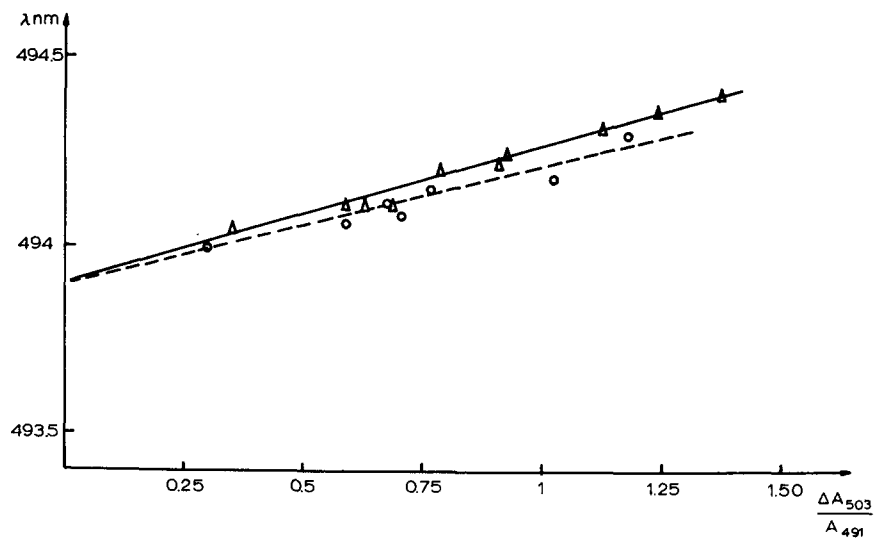

Fig. 6. Plots of the location of the isosbestic point vs. the maximum light-induced change in absorption at different intensities of actinic light for two preparations of *Rps. sphaeroides* G1C chromatophores. Conditions as given in Fig. 1. Triangles, chromatophore preparation after 2×1-min sonication; circles, chromatophores after 2×0.5-min sonication.

An additional boundary to this parameter was set by the CORRELA program. The correlation difference using the parameter values given by DIFSPC was compared with the experimental correlation difference (Fig. 4). Combinations of parameter values giving a minimum least square by DIFSPC seem to fit quite well if $\Delta\lambda_1$ did not exceed 2 nm. The values for $\Delta\lambda_1$ determined experimentally (see above) were within the boundaries set by FITCAR and CORRELA.

The calibration of the membrane potential responsible for the carotenoid band shift was carried out according to the method indicated by Jackson and Crofts [9]; KCl 'pulses' of different concentrations were administered to samples of G1C chromatophores in the presence of 1 μM valinomycin; the resulting absorbance changes were measured in the double beam spectrophotometer described in Materials and Methods. This was done with a number of different preparations. Fig. 5 shows a typical calibration curve. This curve for the larger part was linear, which is in accordance with the results of Jackson and Crofts [9], but levelled off at higher KCl concentrations, as was shown also by Matsuura and Nishimura [26].

In order to use these values of the membrane potential to calibrate the electrochromic shifts, we followed the location of the isosbestic point at different intensities of the actinic light in the same batch of chromatophores. Maximum values of ΔA and of the corresponding $\Delta\lambda$ varied up to 40 % from preparation to preparation. Typical plots are shown in Fig. 6.

The average calibration value determined this way was 137 ± 6 mV per nm shift. The data given in Table I, therefore, represent carotenoid band shifts induced by membrane potential as large as 240 mV.

DISCUSSION

The results presented in this paper clearly show that the carotenoid absorbance changes observed in chromatophores of *Rps. sphaeroides*, G1C are due to electrochromic band shifts. Our analyses have shown that we can roughly divide the carotenoids in two spectrally different pools. Only one of these pools, comprising about 20 % of the total carotenoid content, showed a light-induced red shift of up to 1.75 nm, corresponding to a transmembrane potential of about 240 mV. The 'remaining' carotenoid pool absorbs at wavelengths which are about 5 nm lower than those at which the 'shifting' pool absorbs. Both pools, however, consist of neurosporene.

As shown in Figs. 5 and 6, the light-induced absorbance changes as well as the electrochromic shifts are, in a good approximation, a linear function of the membrane potential (cf. ref. 9). However, because of the non-polarity of the molecule a quadratic dependence should be expected. As pointed out by Schmidt et al. [6, 27], this paradox can be solved by assuming that the carotenoid molecules are subjected to an additional local permanent field. The membrane thickness and the direction of the permanent field with respect to the carotenoid molecules are of particular importance for the calculation of such a permanent field from the experimental data. It seems to us that, considering the chromatophore membrane as the dielectric of a capacitator the protein moiety should be included in the evaluation of the effective membrane thickness; the charges responsible for the membrane potential must remain beyond the boundaries of the chromatophore membrane, as they, according to Witt and Zickler [28], are moving in the solution phase with a velocity determined by the viscosity of the solu-

tion medium. The value of 76 Å, determined by X-ray diffraction on *R. rubrum* chromatophores [29], therefore seems to us a realistic value for the effective membrane thickness at this time.

It is difficult to visualize a permanent electrical field, which is ten times stronger than the light-induced field, to be oriented perpendicularly to the surface of the membrane unless one assumes in addition that the field is strictly localized. Even so, a more plausible assumption would be that the field (which, perhaps, is caused by fixed charges in the vicinity of the heads of the molecules) is more or less parallel to the direction of the carotenoid molecular chain.

The electrochromic shift induced by both a permanent field and an added light-induced field is given by Schmidt [27]:

$$\Delta\nu = \frac{1}{2hc}(\alpha_g - \alpha_e)(F_p \cos\theta' + F_a \cos\theta)^2$$

in which α_g and α_e are respectively the polarizabilities in the ground state and the excited state, F_p is the permanent field and F_a is the light-induced field, and θ' and θ are the angles between the molecular axis and, respectively F_p and F_a. Linear dichroism studies of Breton [30] have shown an angle of 45° for the orientation of the carotenoid molecules in respect to the membrane. If the polarizability of neurosporene is assumed to be 780 $Å^3$ (a value given for the polarizability of crocetindimethyl ester [32]; both crocetindimethyl ester and neurosporene have 9 conjugated double bonds) the permanent field strength has to be $7 \cdot 10^6$ V/cm to account for the light-induced shifts reported presently. Such a field can be shown to cause a displacement of the carotenoid absorption bands by 27 nm, which is about equal to the shift observed when the carotenoids are extracted from the chromatophores [16]. If the permanent field would be oriented perpendicularly to the membrane surface (a possibility which we cannot exclude) its strength must be 10^7 V/cm to account for the observed light-induced shift. The absorption bands then would show a displacement of about 20 nm to the red as compared with the absorption bands of the extracted carotenoids. Thus, it seems that not just the 'shifting' pool but also the 'remaining' pool is influenced by a local permanent field, however, in a different way, possibly as a result of different orientations and/or localizations.

There seems to be no significant electrochromic band broadening; the band widths decreased by no more than 1 % upon lowering the field strength by 40 %. This suggested that the carotenoids, at least those of the shifting population, are rather homogeneously oriented.

A lowering of the oscillator strength by about 1 % is shown by our analyses. The significance of this effect should be established by further studies. In any case, such a change in extinction coefficient cannot explain the distance of 5 nm between the band centre wavelength and the isosbestic point of the difference spectrum of *Rps. sphaeroides*, as suggested earlier by Conjeaud et al. [18].

ACKNOWLEDGEMENTS

We are much indebted to Dr. A. Crofts, University of Bristol, who provided us with cultures of the G1C and the R26 mutants of *Rps. sphaeroides*, to Dr. F. Van Den

Bogaert, Physics Department of the Vrije Universiteit Brussel, who supplied us with the MINUIT minimization program and assisted us in further programming, and to Drs. J. Amesz and B. de Grooth, University of Leiden, who informed us about their unpublished results. The valuable suggestions from Dr. D. Layton, I.C.P. Brussels, and the many fruitful discussions with Dr. L. Slooten of our own laboratory, are much appreciated. We gratefully acknowledge financial support from the Belgian National Bank. One of us (C. Sw.) received a fellowship from the Instituut tot aanmoediging van het Wetenschappelijk onderzoek in Nijverheid en Landbouw.

REFERENCES

1 Smith, L. and Ramirez, J. (1960) J. Biol. Chem. 235, 218–225
2 Clayton, R. K. (1963) Proc. Natl. Acad. Sci. U.S. 50, 583–587
3 Vredenberg, W. J. and Amesz, J. (1966) Biochim. Biophys. Acta 126, 243–253
4 Junge, W. and Witt, H. T. (1968) Z. Naturf. 23, 244–254
5 Emrich, H. B., Junge, J. and Witt, H. T. (1969) Z. Naturf. 24b, 1144–1146
6 Schmidt, S., Reich, R. and Witt, H. T. (1971) in Proc. 2nd Int. Congress Photosynth. (Forti, S., Avron, M. and Melandri, B. A. eds.), pp. 1131–1146, Elsevier, Amsterdam
7 Reich, R., Scheerer, R., Sewe, K.-U. and Witt, H. T. (1976) Biochim. Biophys. Acta 449, 285–294
8 Borisevich, G. P., Kononenko, A. A., Venediktov, P. S., Verkhoturov, V. N. and Rubin, A. B. (1975) Biofizika 20, 254–258
9 Jackson, J. B. and Crofts, A. R. (1969) FEBS Lett. 4, 185–189
10 Jackson, J. B., Saphon, S. and Witt, H. T. (1975) Biochim. Biophys. Acta 408, 83–92
11 Saphon, S., Jackson, J. B. and Witt, H. T. (1975) Biochim. Biophys. Acta 408, 47–57
12 Fleischmann, D. and Clayton, R. K. (1968) Photochem. Photobiol. 8, 287–298
13 Jackson, J. B. and Crofts, A. R. (1971) Eur. J. Biochem. 18, 120–130
14 Amesz, J., 't Mannetje, A. H. and de Grooth, B. G. (1973) Abstr. Symp. Prokaryotic Photosynth. Org. Freiburg, pp. 34–35
15 Okada, M., Murata, M. and Takamya, A. (1970) Plant Cell. Physiol. 11, 518–530
16 Crofts, A. R., Prince, R. C., Holmes, N. G. and Crowther, D (1974) Proc. 3d Congress Photosynth. (Avron, M., ed.), pp. 1131–1146, Elsevier, Amsterdam
17 Holmes, N. G. and Crofts, A. R. (1977) Biochim. Biophys. Acta 459, 492–483
18 Conjeaud, H. and Michel-Villaz, M. (1976) J. Theor. Biol. 62, 1–16
19 Conjeaud, H., Michel-Villaz, M., Vermeglio, A. and Mathis, P. (1976) FEBS Lett. 71, 138–141
20 Joliot, A. P., Delosme, R. and Joliot, A. (1977) Biochim. Biophys. Acta 459, 47–57
21 Amesz, J. and de Grooth, B. G. (1976) Biochim. Biophys. Acta 440, 301–313
22 De Grooth, B. G. and Amesz, J. (1976) in Abstracts of "International Conference on the Primary Electron Transport and Energy Transduction in Photosynthetic Bacteria" Brussels, p. WB1
23 Cohen-Bazire, G., Sistrom, W. R. and Stanier, R. Y. (1957) J. Cell. Physiol. 49, 25–68
24 Labhart, H. (1957) J. Chem. Phys. 27, 957–965
25 Liptay, W. (1969) Angew. Chem. Intern. Edit. 8, 177–188
26 Matsuura, K. and Nishimura, M. (1977) Biochim. Biophys. Acta 459, 483–491
27 Schmidt, S. (1973) Thesis Technical University, Berlin
28 Witt, H. T. and Zickler, A. (1973) FEBS Lett. 37, 307–310
29 Ueki, T., Kataoka, M. and Mitsui, T. (1976) Nature 262, 809–810
30 Breton. J. (1974) Biochem. Biophys. Res. Commun. 59, 1011–1017
31 Clayton, R. K. (1963) in Bacterial Photosynthesis (Gest, H., San Pietro, A. and Vernon, L. P., eds.) p. 498, Antioch Press, Yellow Springs, Ohio
32 Labhart, H., (1961) Helv. Chim. Acta 44, 457–460