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THE CAROTENOID SHIFT IN *RHODOPSEUDOMONAS SPHAEROIDES* THE FLASH INDUCED CHANGE

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SUMMARY

A mutant, *Rhodopseudomonas sphaeroides* G1C, having only one major carotenoid, neurosporene, is described. The spectrum of the carotenoid shift in this mutant is analysed and it is concluded that only 7–11 % of the pigment is involved under conditions of steady-state illumination and that this pigment undergoes a shift of 7 nm.

The spectrum of the carotenoid shift under conditions of multi-flash illumination is examined for changes in shape concordant with a progressive red shift of the pigment with increasing membrane potential; the spectra of the fast change after each of three flashes does not agree well with predictions from a model involving a progressive shift of the pigment, the slow change shows qualitative agreement with such a model but the small size of the signal and the presence of more than one phase makes analysis of this phase more difficult.

No separate pool of carotenoid, that might correspond to that postulated to participate in the carotenoid shift, could be identified by fourth derivative analysis of, or curve fitting to, the spectrum of the neurosporene.

INTRODUCTION

A light induced red shift in the spectrum of the carotenoids of photosynthetic bacteria was first reported in *Rhodospirillum rubrum* by Smith and Ramirez [1]. It has since been shown to occur in many species of photosynthetic bacteria. Empirical observation suggests that the carotenoid change, and similar shifts in the bacteriochlorophyll spectra, are indicators of the electrical potential between the phases separated by the membrane [2–6].

Jackson and Crofts [4] have shown that the amplitude of the absorbance

Abbreviations: MOPS, 3-(*N*-morpholino)propane sulphonic acid; DAD, diamino durene; PMS, phenazine methosulphate; PES, phenazine ethosulphate.

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change, induced by a potassium diffusion potential, in chromatophores from *Rhodopseudomonas sphaeroides*, is linearly related to the size of the membrane potential.

Comparison of the spectrum of the carotenoid shift in *Rps. sphaeroides* with that of the pigment responsible has led workers to suggest that only a proportion (about 10 %) of the pigment is involved in the change [7–9], although the nature of any such pool remains obscure.

A similar red shift in the spectrum of carotenoids and chlorophyll *b* in chloroplasts (the "518 change") has previously been interpreted by Witt [10] as a response to changes in membrane potential [11, 12]. Fowler and Kok [13] and Witt and Zickler [14] have recently shown a direct kinetic correlation between the light induced 518 change in chloroplasts and an electrical change between passive electrodes in the suspending medium which can best be interpreted as related to a membrane potential.

These light induced changes in both photosynthetic bacteria and chloroplasts have very rapid rise times. These and other similarities between the two systems suggest that a common effect is involved and Witt has suggested that the interaction between the electric field and the spectral change in both cases is electrochromic. For an electrochromic mechanism the following relationship between the spectral changes and the electric field has been derived [15]:

$$h\Delta n = -|\vec{\mu}_e - \vec{\mu}_g| F\cos\theta - \tfrac{1}{2}(\alpha_e - \alpha_g)F^2$$

where h is the Planck Constant; Δn is the change in frequency caused by the field; μ_e, μ_g are the dipole moments and α_e, α_g the polarisabilities of the excited and ground states, respectively; F is the electric field; θ is the orientation of the pigment molecules. These authors have also demonstrated that for the carotenoid lutein, in vitro, the relationship between the absorbance change and the electric field is quadratic, the molecule having no permanent dipole moment, and this has led them to suggest that in vivo a permanent field may exist in the membrane producing a pseudo-linear relationship between the absorbance change of the carotenoid and the field. Evidence for this has rested so far on in vitro experiments and on the similarity of the light induced, in vivo, difference spectrum to the first derivative of the absolute absorbance spectrum [15]. Amesz et al. [8], however, have shown that the shape of the light induced difference spectrum in *Rps. sphaeroides* wild type is independent of light intensity, a result that would not be predicted from a simple electrochromic hypothesis, since it would be anticipated that at non-saturating light intensities, increasing the light intensity would generate a greater potential and therefore a greater spectral shift.

We have used a mutant (G1C) of *Rps. sphaeroides* with only a single major carotenoid in an attempt to resolve the nature of the interaction between the electrical field and the carotenoid spectrum.

MATERIALS AND METHODS

An inoculum of the mutant *Rps. sphaeroides* G1C was kindly provided by Dr. V. A. Saunders of this laboratory. The mutant was prepared from *Rps. sphaeroides* wild type by the method previously described [16].

Cells were grown, and chromatophores were prepared as described elsewhere [17] except that the cells were broken in a French Pressure Cell at 2100 $kg \cdot cm^{-2}$.

Spectrophotometric techniques

Spectra were obtained using a spectrophotometer linked to a DEC PDP11/10 computer via a DEC LPS-11 Laboratory Peripheral System as described elsewhere [18] with a modification to permit the use of a Dewar vessel and 1 mm perspex cuvette for the measurement of spectra at low temperature. The Scan Drive Motor on the Hilger D330 monochromator was replaced by a computer driven stepper motor. Spectra were stored on DEC tape and processed by FORTRAN or BASIC language programmes (see below). Actinic light was provided by a 55 W quartz-iodine lamp via a Wratten 88A filter; for spectra under actinic illumination the EMI 9695B photomultiplier was protected by a Corning blue-glass filter (Corning glass no. 9782).

Changes in carotenoid absorbance at a single wavelength were measured in a rapidly responding single beam spectrophotometer [5], but the transients were averaged after having been read into the computer via a Datalab DL905 Transient Recorder (Data Laboratories, Mitcham, Surrey, CR4 4HR, U.K.). Actinic illumination was provided by a saturating Xenon flash (Osram X1E 100) of about 20 μs half-width.

Where indicated, constant redox potential conditions were maintained in an anaerobic cuvette similar to that described by Dutton [19].

Pigment assays

Carotenoids were extracted by the technique of Liaaen-Jensen et al. [20]. Bacteriochlorophyll was assayed after extraction into 7 : 2 acetone/methanol from the absorbancy at 772 nm using an extinction coefficient $E_{mM} = 75$ [21].

Programming

A programme written in PDP11 BASIC was used to generate the difference spectrum resulting from the shift of a proportion of an absolute spectrum by a known number of wavelength units.

Input parameters were the 512 point absolute spectrum, the proportion of the spectrum to be shifted and the distance in terms of wavelength units that that proportion was to be shifted. The difference spectrum so generated could be displayed on an oscilloscope or stored, again as a 512 point spectrum, on DEC tape and compared with the light induced difference spectrum.

Programmes for generating fourth derivatives of spectra were based on the method of Butler and Hopkins [22] and for curve fitting to spectra with mixed Gaussian-Lorentzian bands based on the RESOLV programme of Dr. D. D. Tunnicliff (Shell Development Co., P.O. Box 481, Houston, Texas, U.S.A.) generously made available to us by Professor C. S. French, Dr. J. S. Brown and Mr. G. A. Ford of the Carnegie Institute, Stanford, Calif., U.S.A., (see ref. 23). In order to ensure that the fitted spectra resembled as closely as possible the original spectra, judgement on closeness of fit between the two was based on consideration not only of the absolute absorbance spectra, but also of the fourth derivatives of these spectra.

RESULTS

Carotenoid composition

The carotenoid composition of *Rps. sphaeroides* G1C appears to be similar to

TABLE I

CAROTENOID COMPOSITION OF *RPS. SPHAEROIDES* Ga AND *RPS. SPHAEROIDES* G1C

	% Total carotenoid	
	G1C	Ga
Neurosporene	96	62
Chloroxanthin	–	33
Lycopenes	0.49	0.8
ζ-Carotenes	3.2	3

TABLE II

ELUTION PROFILE FOR CAROTENOIDS OF G1C MUTANT

Separation on 0.5 cm × 15 cm column of Woelm neutral aluminium oxide (Grade 2).

Elution conditions in light petroleum (b.p. 40–60 °C)	Fraction and colour	Absorbance peaks in light petroleum (b.p. 40–60 °C)	Group*
Diethyl ether			
2–5 %	1 orange	466 nm, 437 nm, 412 nm	neurosporene
5–10 %	2 pink	501 nm, 460 nm, 443 nm	lycopene
Methanol			
4 %	3 yellow	425 nm, 400 nm, 380 nm	ζ-carotene (but see text)

* Follows the nomenclature of ref. 20. Thus the neurosporene group refers to carotenoids having the characteristic absorbance spectrum of neurosporene irrespective of the presence or absence of extra hydroxyl groups.

that of the mutants of *Rps. sphaeroides* (M_{55}, EMS_{66}) described by Sunada and Stanier [25]. The major carotenoid (Table I) is neurosporene unlike the Ga mutant which contains significant amounts of the monohydroxylated form of neurosporene chloroxanthin [26]. Neurosporene was differentiated from chloroxanthin on the basis of its elution from an alumina column (Table II) and by its failure to partition into the methanol/water (19 : 1 v,v) layer of a hexane/methanol/water system [27].

The minor carotenoids showed absorption spectra characteristic of lycopene and ζ-carotene. Only one band of each was detected. The exact proportions of lycopene and ζ-carotene varied from preparation to preparation. Recent work has suggested that the pigment previously identified as ζ-carotene (7,8,7′,8′-tetreahydrolycopene) may in fact be 7,8,11,12-tetrahydrolycopene which has similar spectral properties [28].

Carotenoid shift

The simpler carotenoid composition of this mutant has been exploited in an investigation of the nature of the interaction causing the carotenoid shift. Preliminary experiments showed that the carotenoid shift of the G1C mutant behaved similarly to that of the wild type and Ga mutants of *Rps. sphaeroides*. The magnitude of the change

showed a linear relationship with a transmembrane diffusion potential produced by K^+ pulses in the presence of valinomycin [4, 29]. The carotenoid shift induced by a saturating flash was also similar to that found in the wild type, both in kinetics and in the effects of anitbiotics and uncouplers (ref. 29 and Holmes, N. G. and Crofts, A. R., unpublished observation).

A comparison of the light induced difference spectrum, in the carotenoid region, with computer generated curves obtained by shifting a proportion of the absolute spectrum (Fig. 1), demonstrates that 11 % of the pigment has undergone a shift to the red of about 7 nm, in agreement with the results of Amesz and Vredenberg and Okada et al. [7, 9] obtained with *Rps. sphaeroides* wild type. The proportion of the total carotenoid absorbance that undergoes a shift has varied between 7 and 11 % in different preparations.

Using these values for the parameters of the shift, a model of the carotenoid shift showing increasing magnitudes of the shift, has been constructed on the assumption that it is an electrochromic process. Such a model predicts a change in shape of the difference spectrum of the carotenoid shift, with increasing magnitude of the change over the range of sizes of change induced in the light. Each increment of the shift alters both the position of the peaks of the difference spectra and the position at which the difference spectra cross the base line. In order to test the hypothesis that the carotenoid shift is an electrochromic phenomenon we have used this shift of the zero point in the difference spectra as an indicator of the expected shape of the flash induced spectra. In order to maximise the signal to noise ratio of the flash induced changes only the change associated with the long wavelength peak of the carotenoid spectrum has been investigated.

These experiments were carried out at a redox potential of $+125 \pm 10$ mV, in order to maximise the change, and in the presence of antimycin in order to eliminate the slow phase of the change and simplify the kinetics [30].

In Fig. 2 are shown the kinetics of the carotenoid shift in the presence of antimycin. They are seen to be similar to the kinetics of the Ga mutant under the same

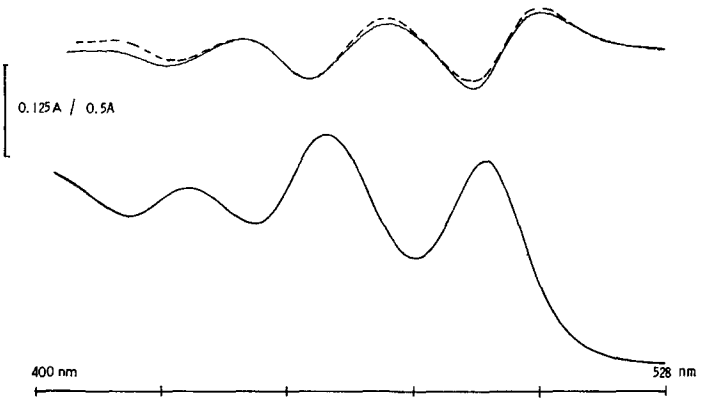

Fig. 1. Light induced and fitted carotenoid shift spectra. Chromatophores of *Rps. sphaeroides* G1C, suspended to a concentration of 28 $\mu g \cdot ml^{-1}$ bacteriochlorophyll in 50 mM MOPS, 50 mM KCl, pH 7.0. Upper trace: solid line, light minus dark difference spectrum; dashed line, modelled shift of 11 % of spectrum by 7 nm. Lower trace: absolute absorbance spectrum.

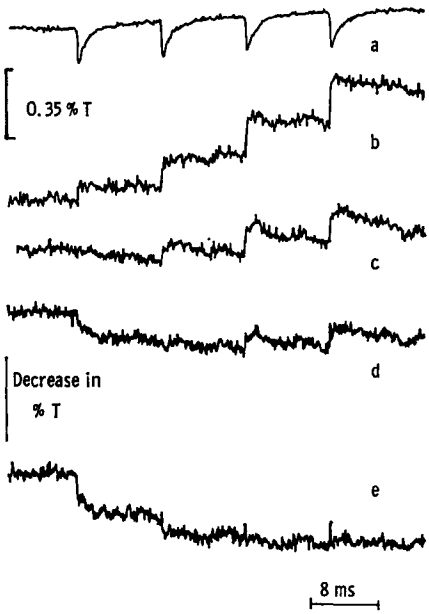

Fig. 2. Kinetics of the carotenoid change. Kinetics of the P605 change and corrected kinetics of the carotenoid change in *Rps. sphaeroides* G1C. Chromatophores (37 $\mu g \cdot ml^{-1}$ bacteriochlorophyll) were suspended in 50 mM MOPS, 50 mM KCl, pH 7.0 with 10 μM each of phenazine methosulphate (PMS), phenazine ethosulphate (PES), diamino durene (DAD), 1,2-naphthoquinone and 1,2-naphthoquinone-4-sulphonate as redox mediators. The redox potential was maintained at $E_h = +125 \pm 10$ mV. Each trace is an average of 64. Traces are changes at (a) 605 nm, multiplied by -0.4 (see text); (b) 493.4 nm $-$ (a); (c) 493.7 nm $-$ (a); (d) 494 nm $-$ (a); (e) 494.2 nm $-$ (a).

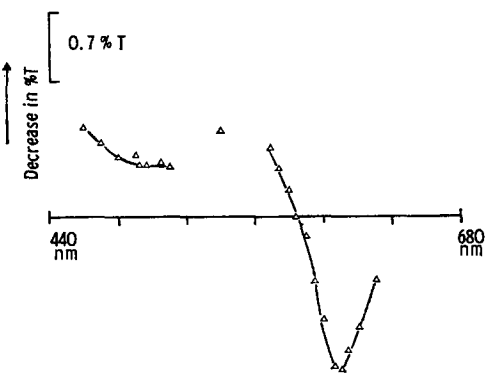

Fig. 3. Spectrum of rapid flash induced absorbancy change in *Rps. sphaeroides* R26. Chromatophores were suspended to a concentration of 33 $\mu g \cdot ml^{-1}$ bacteriochlorophyll in the presence of 10 μM each of PMS, PES, DAD. $E_h = +100 \pm 15$ mV. Each trace was an average of 8 taken at a frequency of 0.2 Hz.

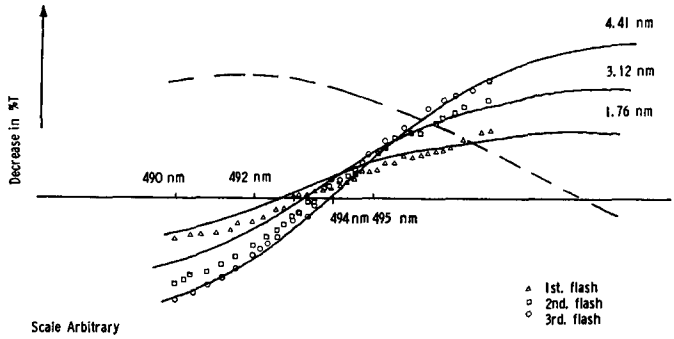

Fig. 4. Corrected spectrum of the carotenoid change. Points from experiments similar to Fig. 2, except that 1 μM antimycin was present to eliminate the slow phase, are compared with the spectrum expected from a linear carotenoid shift (see text). Each trace was an average of 8. Solid lines represent predicted change (see text). Dashed line shows position of long wavelength peak of carotenoid spectrum.

conditions, showing an initial phase, faster than the rise time of the instrument, associated with charge separation in the photochemical reactions, and a second slower phase corresponding kinetically to cytochrome c_2 photooxidation [31]. It has been found necessary to make a correction for absorbance changes observed in this region of the spectrum in the R26 mutant of *Rps. sphaeroides* which contains no coloured carotenoids. The flash induced changes observed with chromatophores from R26 have the spectrum shown in Fig. 3, which is similar to that observed on oxidation of isolated lauryl dimethylamine oxide reaction centres of this strain [32]. It would seem reasonable to suppose that a change as intimately linked to the reaction centre as this would be found also in the G1C mutant, although masked there by the carotenoid shift. Correction has therefore been made for this transient by subtracting a suitable proportion of the reaction centre change measured at 605 nm from the flash induced changes measured in the region of the carotenoid change. In Fig. 4 the observed flash induced spectrum is seen compared with the computer generated shift that would be predicted from a simple electrochromic hypothesis. The magnitude of the computed shift is

Fig. 5. Carotenoid shift induced by continuous and multi-flash illumination. Chromatophores of *Rps. sphaeroides* G1C were suspended to a concentration of 28 $\mu g \cdot ml^{-1}$ bacteriochlorophyll in 50 mM MOPS, 50 mM KCl, pH 7.0. Measuring wavelength 503 nm.

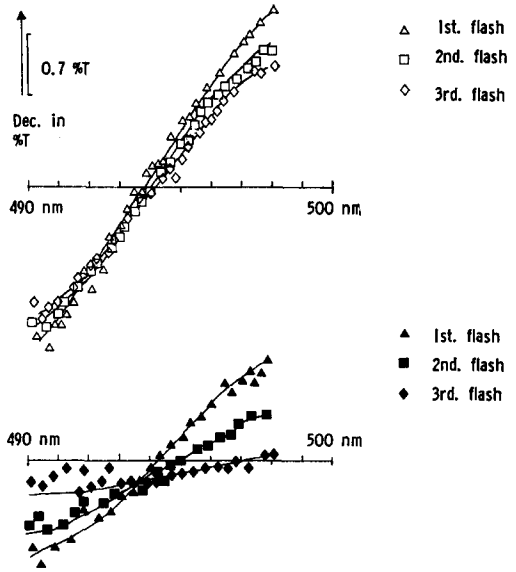

Fig. 6. Spectra of the flash induced carotenoid shift in the absence of antimycin A. Chromatophores of *Rps. sphaeroides* G1C were suspended to a concentration of 18 $\mu g \cdot ml^{-1}$ bacteriochlorophyll in 50 mM MOPS, 50 mM KCl, pH 7.0 in the presence of 10 μM each of PMS, PES, DAD, 1,2-naphthoquinone and 1,2-naphthoquinone-4-sulphonate. Redox potential $E_h = +120 \pm 15$ mV. Top: fast phase (0.4 ms after flash). Bottom: slow phase (10 ms after flash).

based on the total absorbance change produced by the three flashes, assuming that the wavelength change is proportional to the size of the absorbance change at a fixed wavelength, and hence to membrane potential as is suggested by the work of Jackson and Crofts [4]. Fig. 5 compares the changes elicited by continuous and multi-flash illumination, measured at 503 nm and in the absence of antimycin (and thus showing the slow phase of the flash induced change), from which it can be seen that the total change induced by three flashes is not as large as the change under continuous illumination, the size of the computed shift has therefore been adjusted for this.

From the shape of the corrected traces (Fig. 2) it can be seen that successive flashes shift the spectrum further to the red. However, as the spectrum (Fig. 4) demonstrates, the shift does not correspond well to that predicted from a linear electrochromic effect, the initial flash provoking a greater shift than would be anticipated, and further flashes a smaller shift.

In chromatophores in the absence of antimycin, the carotenoid change shows an additional slower phase, interpreted by Jackson and Crofts as representing a second site of electrogenic electron flow [5]. The fast phase shows a similar spectrum to the fast phase in the presence of antimycin and little movement of the zero point of the spectrum can be seen. However, the slow phase, which is a mixture of the antimycin insensitive fast-slow phase and a slow phase associated with electron flow through an antimycin sensitive site shows a shift in the zero point of its spectrum with increasing flash number (Fig. 6). It is not possible to produce a simple quantitative model for this situation similar to that described above, because of the mixing of two phases with seemingly different behaviours.

Analysis of the spectrum of neurosporene in vivo and in vitro

If the carotenoid shift is indeed a change in which increasing membrane potential causes a pool of the carotenoid to shift by increasing amounts then a part of the carotenoid (that involved in the shift) apparently differs, in some way, from the rest of the carotenoid. To try to detect such a pool we have therefore undertaken a detailed comparison of the in vitro and in vivo spectra of neurosporene.

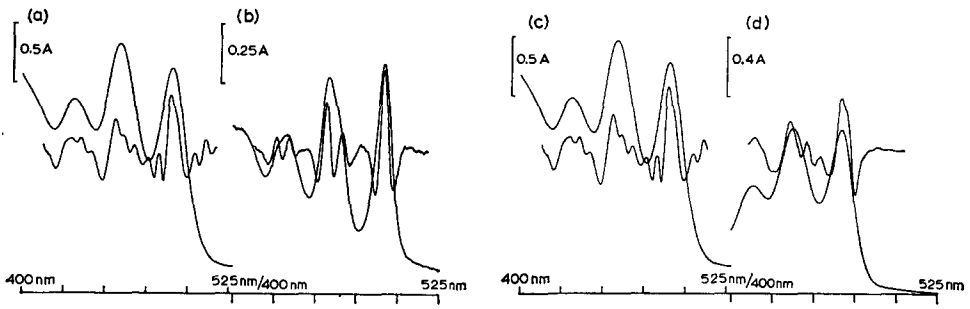

Fig. 7. Comparison of fourth derivatives of spectra of carotenoids. (a) In vivo carotenoid spectrum at room temperature, dark. Chromatophores of *Rps. sphaeroides* G1C were suspended to a concentration of 46 $\mu g \cdot ml^{-1}$ bacteriochlorophyll in 50 mM MOPS, 50 mM KCl, pH 7.0 in the presence of 1 $\mu g \cdot ml^{-1}$ oligomycin to slow the rate of decay of the carotenoid shift. (b) In vivo carotenoid spectrum at 77 °K, dark. Chromatophores of *Rps. sphaeroides* G1C were suspended to a concentration of 70 $\mu g \cdot ml^{-1}$ bacteriochlorophyll in 50 mM MOPS, 50 mM KCl, pH 7.0 and the suspension mixed with an equal volume of glycerol before freezing in liquid nitrogen. (c) In vivo carotenoid spectrum at room temperature, light. As (a) but under continuous illumination. (d) In vitro carotenoid spectrum. Purified neurosporene in light petroleum (b.p. 40–60 °C). Differentiating intervals for all spectra: 4.0, 3.5, 3.25 and 3.0 nm.

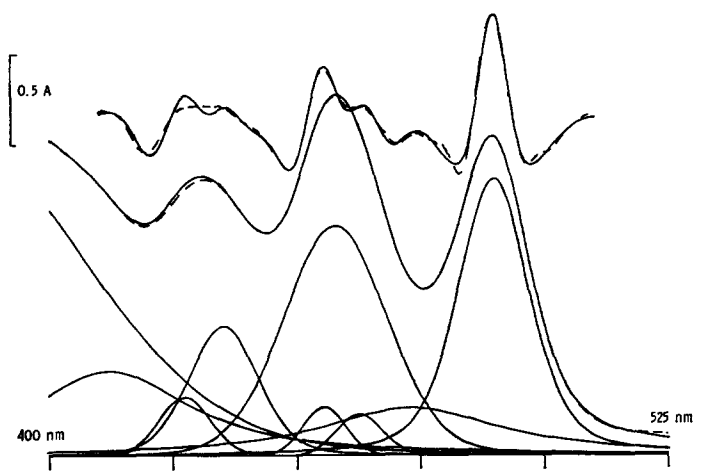

Fig. 8. Components of the carotenoid region of the spectrum of *Rps. sphaeroides* G1C at room temperature. Chromatophores of *Rps. sphaeroides* G1C were suspended to a concentration of 37 $\mu g \cdot ml^{-1}$ bacteriochlorophyll in 50 mM MOPS, 50 mM KCl, pH 7.0. Dashed line indicates original spectrum, solid line indicates fitted spectrum. Bottom: absolute spectrum with component bands of fitted spectrum and their envelope. Top: fourth derivatives of spectra (differentiating intervals: 4.0, 3.5, 3.25 and 3.0 nm.)

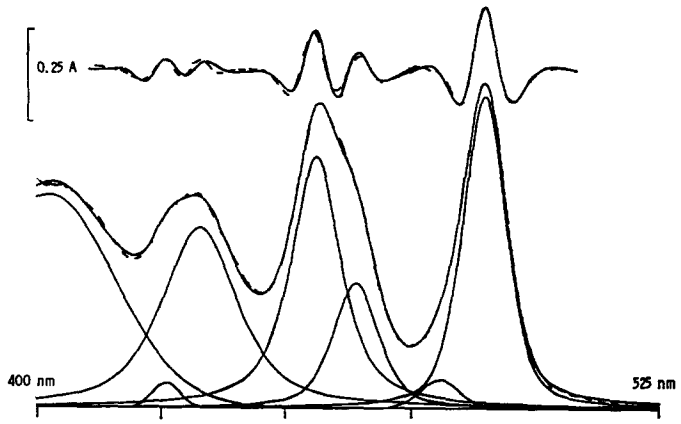

Fig. 9. Components of the carotenoid region of the spectrum of *Rps. sphaeroides* G1C at 77 °K. Conditions as Fig. 7b. Dashed line indicates original spectrum, solid line fitted spectrum. Bottom: absolute spectrum with component bands of fitted spectrum and their envelope. Top: fourth derivatives of spectra (differentiating intervals: 4.0, 3.5, 3.25 and 3.0 nm).

A comparison of the fourth derivatives of the spectra of G1C chromatophores and of isolated neurosporene in light petroleum (b.p. 40–60 °C), shown in Fig. 7a, d revealed no significant differences beyond the shift of the in vivo spectrum some 23 nm to the red. The fourth derivatives of both spectra are complex but corresponding bands show similar features. Of particular note is the longest wavelength peak, where in chromatophores the least interference from the Soret band of bacteriochlorophyll *a* would be expected.

Similarly, no distinctive difference could be seen in the fourth derivative of the spectrum of chromatophores in the light, although the latter spectrum is, overall, shifted slightly to the red. At 77 °K the carotenoid spectrum in chromatophores is

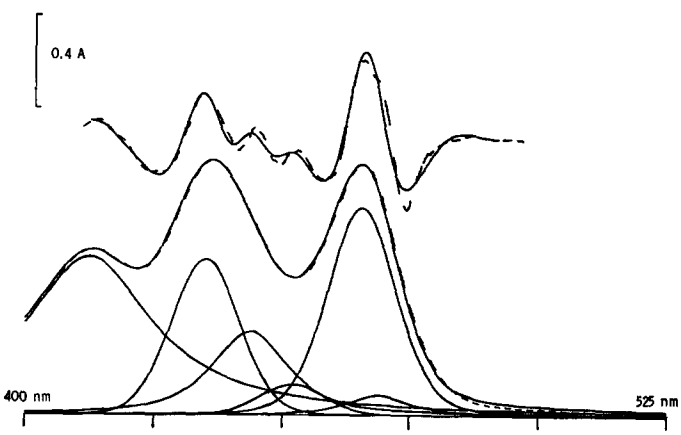

Fig. 10. Components of the spectrum of isolated neurosporene. Conditions as Fig. 7d. Dashed line indicates original spectrum, solid line fitted spectrum. Bottom: absolute spectrum with component bands of fitted spectrum and their envelope. Top: fourth derivatives of spectra (differentiating intervals: 4.0, 3.5, 3.25 and 3.0 nm).

TABLE III

PARAMETERS OF BANDS TO FIT CAROTENOID ABSORBANCE SPECTRA IN VIVO AND IN VITRO

Band centres are expressed to the nearest 0.5 nm.

	Chromatophores of *Rps. sphaeroides* G1C (room temperature)			
Band	Centre (nm)	Half-width (nm)	Maximum absorbance	
			Gaussian (*A*)	Lorentzian (*A*)
1	381.5	64	1.569	0.157
2	412.5	39	0.069	0.399
3	428	12	0.322	0.0
4	436	17	0.58	0.136
5	457	10	0.269	0.0
6	459	25	1.158	0.111
7	464	11	0.229	0.0
8	474.5	44	0.003	0.262
9	491.5	17	0.928	0.6
	Chromatophores of *Rps. sphaeroides* G1C (77 °K)			
Band	Centre (nm)	Half-width (nm)	Maximum absorbance	
			Gaussian (*A*)	Lorentzian (*A*)
1	401.5	33	0.575	0.0
2	412	9	0.013	0.015
3	427	9	0.112	0.0
4	434	18	0.144	0.313
5	457	12	0.124	0.518
6	464.5	11	0.063	0.304
7	491.5	11	0.352	0.497
	Neurosporene in light petroleum (b.p. 40–60 °C)			
Band	Centre (nm)	Half-width (nm)	Maximum absorbance	
			Gaussian (A)	Lorentzian (*A*)
1	413	31	0.0	0.706
2	436	16	0.65	0.05
3	445	18	0.104	0.276
4	453	15	0.125	0.015
5	467	16	0.748	0.18
6	470	15	0.002	0.09

sharpened up and features previously visible only in the fourth derivative of the room temperature spectrum can be seen in the absolute absorbance spectrum (Fig. 7b). No separation of the carotenoids into distinct pools can, however, be seen.

The second analytical technique used was that of resolving the carotenoid bands into mathematically defined components of mixed Gaussian and/or Lorentzian character. Such a deconvolution does not lead to the identification of distinct species of carotenoid in the same way that the deconvolution of the red bands of chlorophyll *a*

in vivo does (see ref. 23). The main peaks of carotenoids in the 400–600 nm region of the spectrum represent a single absorbance band with vibrational fine structure [33]. By comparison of the components necessary to produce a synthetic spectrum, with a good fit to the original spectrum it is, however, possible to make a comparison between spectra.

It is possible to obtain many fits of equal mathematical validity and it is necessary, therefore, to impose restrictions on the solutions allowed. The fourth derivatives of spectra have been used not only as a guide to the wavelengths of the centres of the bands involved, but also as a check on the nearness of fit obtained. The minimum number of components required has been used.

The results obtained from the resolutions of the absolute spectra of chromatophores of *Rps. sphaeroides* G1C at room temperature and at liquid nitrogen temperature can be seen in Figs. 8–10. Table III shows the bands that were used to fit these spectra.

It can be seen that although there is not an exact correspondence of relative position and half-width between the bands of isolated neurosporene and neurosporene in vivo, the spectrum in vivo is no more complex than that in vitro. In particular it should be noted that the long wavelength carotenoid peak can be fitted with the assumption that it consists of a single band. It was found necessary to restrict the allowed variation of the wavelength of maximum absorbance in order to obtain a good fit to the fourth derivative of the spectrum.

DISCUSSION

These results would seem to indicate that a small part, but not all, of the light generated shift in the carotenoid spectrum can be accounted for by a shift to the red which increases with increasing change and may therefore be an electrochromic effect. Although it has not been possible with our present apparatus to resolve sufficiently accurately the spectrum of the antimycin insensitive intermediate phase, there appears to be a difference in behaviour between the fast phase and the slow antimycin sensitive phase. The fast phase appears to have only a small progressive shift and the effect is sufficiently close to the limits of our technique to make us wary of making definite statements about the electrochromic nature (or otherwise) of this change. The zero point of the slow phase, however, does shift measurably to the red and thus behaves qualitatively as an electrochromic effect. The small size of the slow phase makes it difficult to give a quantitative estimation of the change but the total carotenoid shift after the first flash is of the order of 4 nm and that after the second flash of 6 nm, comparable with that associated with the total magnitude of the steady-state light induced change. A true progressive shift of the spectrum would not be expected to show a single isosbestic point; the data of Fig. 6 may be interpreted as showing either a single isosbestic displaced from the base line, or the lack of an isosbestic expected from the progressive shift model.

Several sorts of explanation may be offered to explain the difference between the fast and the slow phase. It is possible that some unidentified change, which does not appear with the blue-green R26 mutant (and is therefore not corrected for by our treatment) is contributing to the fast phase of the carotenoid change so as effectively

to restrain the movement of the zero point of the fast phase. Alternatively, a slow component of the kinetics due to a broad absorbance change distinct from the carotenoid change, may displace the spectrum of the slow phase from the base line.

Other explanations must envisage that the two phases are different phenomena. This is less attractive from a number of points of view. Thus the effects of antibiotics on the 515 nm change in chloroplasts, and on all phases of the carotenoid shift in chromatophores are essentially similar [4, 5, 10]. The 515 nm change in chloroplasts does not show the multiphasic rise kinetics of the carotenoid shift over comparable time ranges (but see ref. 34) yet shows a similar response to ionophorous antibiotics and other modifiers of the transmembrane electrical potential as does the carotenoid shift. Furthermore, there is good evidence for the existence of two electrogenic reactions in the cyclic electron transport of *Rps. sphaeroides*. The effects of antimycin on, and the kinetic compatibility between, the flash induced changes of cytochromes *b* and c_2, and the slow phase of the carotenoid change, as well as slow phases of rapid H^+-uptake [35] are all compatible with a second site distinct from that associated with the photochemical reaction. Both sites might be expected to have a similar effect on the carotenoid spectrum if the change reflects the transmembrane field.

For these reasons, and because these measurements are made at the limits of our apparatus, we would caution against taking these results as providing unambiguous support for, or evidence against, the electrochromic hypothesis. It is clear from these results that only a proportion of the carotenoids undergoes a change and that only a part if any of the change represents a progressive shift. If the change was electrochromic in nature, and involved a progressive shift, it would be necessary to suggest that the change of only a proportion of the pigment must indicate the existence of a special environment or configuration for that proportion. If the change is not electrochromic then the mechanism need not involve a proportion of the pigment in a distinct environment.

The use of this mutant has demonstrated that the fraction undergoing the shift cannot simply be a discrete chemical species. Our failure to detect a pool of neurosporene spectrally distinct from the bulk of the neurosporene, and the similarity in shape of the spectrum of the neurosporene in vivo and in vitro suggests that if a fraction of the pigment does occupy a distinct environment this environment cannot significantly alter the spectral properties of the neurosporene.

Recently, however, de Grooth and Amesz [24] have reported a shift of the zero point of the carotenoid shift in chromatophores of *Rps. sphaeroides* wild type under conditions (−30 °C and in the presence of phenazine methosulphate) where very large carotenoid shifts can be seen. They have interpreted this as indicating that only a part of the carotenoid complement absorbing at a slightly longer wavelength undergoes an electrochromic shift. Their results suggest a shift of 0.25 nm for each reaction centre transporting one electron across the membrane; such a shift is not inconsistent with the flash induced spectra reported above.

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