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SHIFTS OF BACTERIOCHLOROPHYLL AND CAROTENOID ABSORPTION BANDS LINKED TO CYTOCHROME *c*-555 PHOTOOXIDATION IN *CHROMATIUM*

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SUMMARY

Shifts in the absorption bands of bacteriochlorophyll and carotenoids in *Chromatium vinosum* chromatophores were measured after short actinic flashes, under various conditions. The amplitude of the bacteriochlorophyll band shift correlated well with the amount of cytochrome *c*-555 that was oxidized by P_{870}^+ after a flash. No bacteriochlorophyll band shift appeared to accompany the photooxidation of P_{870} itself, nor the oxidation of cytochrome *c*-552 by P_{870}^+. The carotenoid band shift also correlated with cytochrome *c*-555 photooxidation, although a comparatively small carotenoid shift did occur at high redox potentials that permitted only P_{870} oxidation.

The results explain earlier observations on infrared absorbance changes that had suggested the existence of two different photochemical systems in *Chromatium*. A single photochemical system accounts for all of the absorbance changes.

Previous work has shown that the photooxidations of P_{870} and cytochrome *c*-555 cause similar changes in the electrical charge on the chromatophore membrane. The specific association of the band shifts with cytochrome *c*-555 photooxidation therefore argues against interpretations of the band shifts based on a light-induced membrane potential.

INTRODUCTION

Illumination of photosynthetic bacteria causes optical absorbance changes that are associated with the oxidation of a reactive bacteriochlorophyll complex, P_{870} (refs. 1, 2). Although P_{870} comprises only a small fraction of the total bacteriochlorophyll in the chromatophore membrane, it plays a central role in the primary photochemical reaction of photosynthesis[3,4].

Abbreviations. CCCP, carbonylcyanide-*m*-chlorophenylhydrazone, *E*, redox potential, E_m, the midpoint of a symmetrical redox titration, PMS, *N*-methylphenazonium methosulfate.

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In 1963, Clayton[5] discovered that illumination of *Rhodopseudomonas spheroides* also resulted in a bathychromic shift of an absorption band at 850 nm. The band shift occurred only in fresh cell suspensions and it was most pronounced under reducing conditions, whereas P_{870} oxidation occurred in aged suspensions under oxidizing conditions. Clayton[5] therefore attributed the band shift to the reduction of a second reactive bacteriochlorophyll species, which he called B. Later studies by Vredenberg *et al.*[6,7] revealed that similar band shifts occur in other photosynthetic bacteria, including *Chromatium vinosum* (*Chromatium* strain D) By integrating the light-*minus*-dark difference spectra over the wavelength interval from 800 to 900 nm, Vredenberg and Amesz[7] generated spectra that were similar to the absorption spectra of the bulk, light-harvesting bacteriochlorophyll. Further calculations suggested that a large fraction of the total bacteriochlorophyll molecules participated in the band shift, and that the absorption of a single quantum influenced the spectra of 10 to 15 molecules. For these reasons, Vredenberg and Amesz[7] attributed the band shift to the light-harvesting bacteriochlorophyll, rather than to a special, reactive bacteriochlorophyll complex

Cusanovich *et al.*[8] subsequently revived the idea that infra-red absorbance changes in *C. vinosum* revealed the oxidation or reduction of a reactive bacteriochlorophyll complex distinct from P_{870} The basis for their suggestion was that an absorbance increase at 905 nm appeared on continuous illumination at comparatively low redox potentials, whereas P_{870} photooxidation appeared only at higher potentials. Schmidt and Kamen[9] recently have offered the same analysis of a light-induced absorbance decrease at 836 nm.

A different perspective on the infrared absorbance changes emerges from the studies of Jackson and Crofts[10,11] on *Rps spheroides*, and from the work of Junge and coworkers[12,13] on chloroplasts. These investigators have interpreted shifts in the absorption bands of endogenous carotenoids as an indicator of the formation of a membrane potential. The key observation supporting this interpretation is that uncouplers and certain ionophores cause a rapid reversal of the spectral shifts. By facilitating the flow of ions across the membrane, these agents would dissipate a membrane potential. Jackson and Crofts[10] also have demonstrated that the creation of a membrane potential in the dark generates absorbance changes that are similar to those that result from illumination. The effects of uncouplers, ATP, and pyrophosphate on the carotenoid band shifts[14,15], and the intimate relationship between the band shifts and delayed luminescence[14,16,17] suggest that the band shifts reflect a high energy state which is critical to the mechanism of photophosphorylation. Most of the work aimed at investigating this hypothesis has concerned shifts in the spectra of carotenoids, but Fleischman and Clayton[14] and Vredenberg *et al.*[6] have reported that the bacteriochlorophyll band shifts in *Rps. spheroides* are similar in many respects Because the carotenoid band shift in *C. vinosum* has a comparatively small amplitude, it generally has escaped notice (*cf* refs 6, 17).

All of the studies[5,8,9] which have suggested infrared absorbance changes arising in a photochemical reaction center distinct from P_{870} have relied on continuous actinic illumination. This reliance frequently introduces considerable complexity. P_{870} photooxidation may be apparent only at high redox potentials because a rapid reduction of P_{870}^{+} occurs if the potential is low enough so that the membrane-bound cytochromes are initially in the reduced form[3,18-21]. The cytochromes can be

reduced in turn by exogenous redox buffers. Continuous illumination therefore sets up a steady state, in which the concentration of P_{870}^+ depends on the redox potential in a complex manner. The appearance of some of the infrared absorbance changes only at lower redox potentials suggested to us, not that the absorbance changes originate in a reaction center other than P_{870}, but rather that they accompany the oxidation of cytochromes by P_{870}^+. The present paper explores this possibility, using short actinic flashes at controlled redox potentials.

MATERIALS AND METHODS

C. vinosum was grown as described previously[21]. For the preparation of chromatophores, cell suspensions in 0.4 M sucrose containing 0.1 M Tris–HCl, pH 7.5, were sonicated at 0–10 °C (Branson Instruments LS-75 operated at 5 A for 2 periods of 1 min, 30 s apart) and then centrifuged for 15 min at 27 000 $\times g$. The supernatant was recentrifuged for 90 min at 105 000 $\times g$ to sediment the chromatophore fraction, which was resuspended in fresh sucrose-buffer mixture and kept at 0 °C for use within 1 day. Longer storage led to a decline in the amplitude of the light-induced bacteriochlorophyll band shift. Bacteriochlorophyll was measured spectrophotometrically after extraction into acetone–methanol (7 · 2, v/v)[23], or into methanol alone in some experiments.

Absorbance changes following flashes from two Q-switched ruby lasers and a Xe lamp were measured essentially as described in refs 21 and 22. Techniques for measuring and controlling the redox potential during the absorbance measurements also were standard[21]. The infrared absorbance measurements employed a photomultiplier with gallium arsenide photocathode (RCA C 31034). Signals from the photomultiplier and preamplifier were digitized and stored in a Biomation Model 802 transient recorder, and then transfered to a digital computer of average transients of our own design. A Biomation Model 610 transient recorder was used for the measurements of Fig 10. The infrared absorbance measurements of Fig. 9 employed a United Detector Technology PIN 25 photodiode, rather than a photomultiplier.

RESULTS

Fig. 1 shows typical measurements of flash-induced band shifts in *C. vinosum* chromatophores. For comparison, the figure also shows measurements of cytochrome *c*-555 photooxidation in the same samples. Spectra of the band shifts are shown in the upper parts of Figs 2 and 3

Study of the decay kinetics after a flash allows one to distinguish absorbance changes due to the band shifts from those due to P_{870} and the cytochromes. In the absence of agents that increase membrane conductivity, both the bacteriochlorophyll and the carotenoid band shifts decay with a half-time of about 1 s. The decay kinetics differ in detail from the reduction kinetics of cytochrome *c*-555 (Fig. 1A). Lowering *E* in the presence of *N*-methylphenazonium methosulfate (PMS) greatly accelerates the cytochrome reduction, with no concomitant recovery or enhancement of the bacteriochlorophyll or carotenoid band shift (Figs 1C and 1D). In the presence of K^+, valinomycin causes a rapid reversal of the band shifts (Figs 1B, 2–4) with no effect on either the cytochrome recovery kinetics (Fig. 1B), or the P_{870} absorbance changes

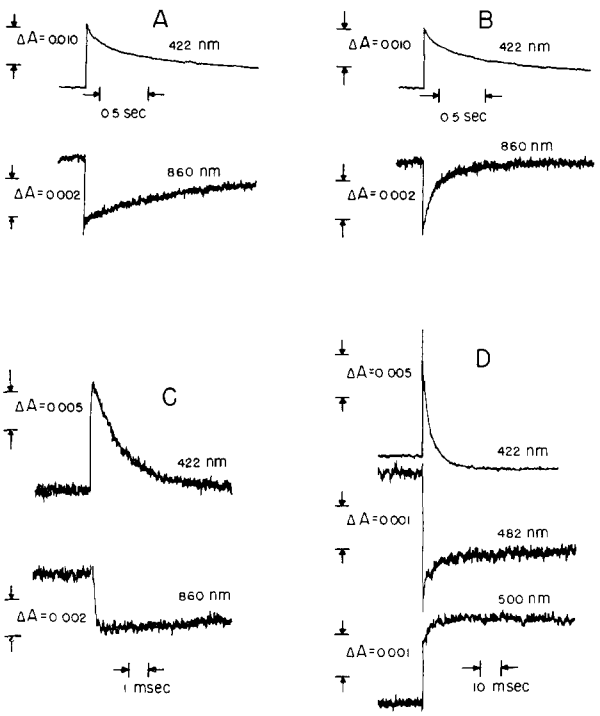

Fig 1. Decay kinetics of the band shifts, compared with the kinetics of reduction of photooxidized cytochrome *c*-555. Upward deflections represent absorbance decreases (A) Bacteriochlorophyll, 27 μM, 0 1 M potassium phosphate, pH 7 7, 0 4 M sucrose No redox buffers added, E (apparent) = 105$\pm$17 mV. Saturating Xe actinic flash The traces are averages, computed from 10 separate measurements at 860 nm (bacteriochlorophyll band shift) and 10 at 422 nm (cytochrome *c*-555) Baseline in absence of flash has been subtracted (B) Same as A, except *plus* 0.2 μM valinomycin (C) Approximately 30 μM bacteriochlorophyll, 0 1 M Tris–HCl, pH 7 5, 0 4 M sucrose, 100 μM PMS, E = 91$\pm$2 mV. Averages of 20 measurements at 860 nm and 10 at 422 nm (D) Conditions as in C Averages of 5 measurements at 422 nm, 10 at 482, and 10 at 500 nm E = 56$\pm$8 mV. The part of the initial absorbance change at 482 that recovers rapidly probably is due to cytochrome *c*-555 Its spectrum is featureless in this region At 500 nm, it appears as a secondary absorbance decrease following the initial change The lower part of Fig. 2 shows a spectrum of the portion that recovers more slowly.

(Fig. 3). Valinomycin has no effect if Na^+ replaces K^+ (Fig 4), as one would expect from the ion specificity of the antibiotic[24]. The rate constant for the accelerated recovery in the presence of K^+ is approximately proportional to the valinomycin concentration (Fig. 4). The uncoupler carbonylcyanide-*m*-chlorophenylhydrazone (CCCP), which increases proton conductivity across the chromatophore membrane, has a similar effect, except that the proportionality constant is smaller by a factor of about 100 (Fig. 4). These results agree well with the observations of Jackson and Crofts[11] on the carotenoid shifts in *Rps. spheroides.*

The addition of 0.5 mM ADP under phosphorylating conditions (0.4 M sucrose, 0.1 M Tris–HCl, pH 7.5, 30 mM potassium phosphate, 5 mM $MgCl_2$, 50 μM PMS) had no significant effect on the decay kinetics of the bacteriochlorophyll band shift. The decay of the bacteriochlorophyll band shift after three closely-spaced

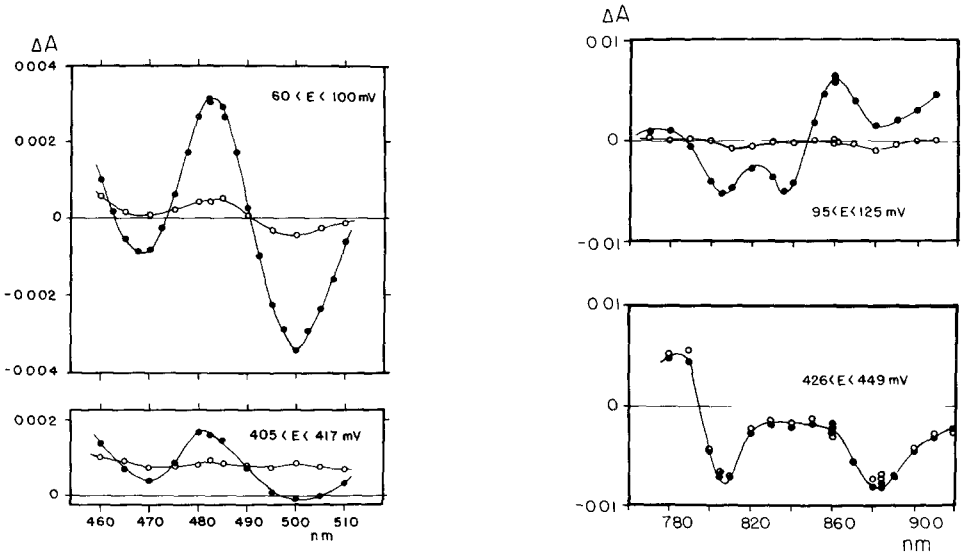

Fig. 2. Visible absorbance changes caused by single Xe flashes. 34 μM bacteriochlorophyll, 0.1 M potassium phosphate, pH 7.7, 0.4 M sucrose, 50 μM PMS. Upper figure: $E = 80 \pm 20$ mV. Lower figure: 25 μM potassium ferrocyanide and 250 μM potassium ferricyanide were added, and E was adjusted to 411 ± 6 mV by the addition of $Na_2S_2O_4$. The measurements include only absorbance changes that remain at approximately 100 ms after the flash. ●, no other additions. ○, *plus* 2 μM valinomycin.

Fig. 3. Infrared absorbance changes caused by single Xe flashes. 40 μM bacteriochlorophyll, 0.1 M potassium phosphate, pH 7.7, 0.4 M sucrose, 50 μM PMS. Upper figure: $E = 110 \pm 15$ mV. Lower figure: E raised to 438 ± 12 mV by the addition of 100 μM potassium ferricyanide. The measurements include only absorbance changes that remain at approximately 20 ms after the flash. ●, no other additions. ○, *plus* 2 μM valinomycin.

flashes was no more sensitive to ADP, P_i or Mg^{2+} than was that after a single flash.

Figs 2 and 3 show spectra of the carotenoid and bacteriochlorophyll band shifts that result from single flashes in two different redox potential regions. For the upper part of each figure, the redox potential was poised so that cytochrome *c*-555 was extensively reduced before the flash and was able to reduce P_{870}^+ rapidly after the flash. The figures show only absorbance changes that remain after this step. Absorbance changes due to cytochrome *c*-555 oxidation do not make a major contribution to the carotenoid shift spectrum in the upper part of Fig. 2, because reduced PMS was present to reduce the cytochrome rapidly (see Fig. 1D).

The spectrum in the upper part of Fig. 3 shows all of the features that Cusanovich *et al.*[8] and Schmidt and Kamen[9] have attributed to a reactive bacteriochlorophyll complex other than P_{870}: absorbance increases at 860 and 905 nm and decreases at 810 and 836 nm. The spectra that Vredenberg *et al.*[6] described for suspensions of intact cells showed the same features, except for the absorbance decrease at 836 nm. We found the relative height of the 836 nm peak to vary somewhat among different chromatophore preparations (see Fig. 9).

For the lower parts of Figs 2 and 3, the redox potential was poised at a level substantially above the E_m of cytochrome *c*-555. Because the cytochrome was unable

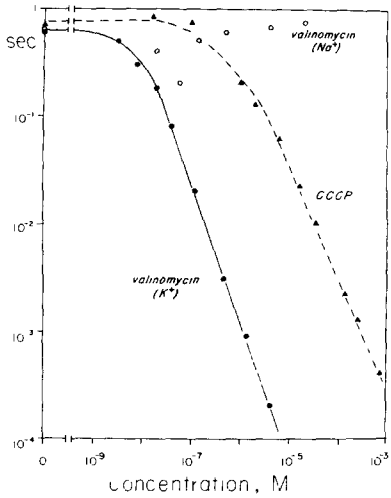

Fig. 4 Half-time of the decay of the bacteriochlorophyll band shift (measured at 860 nm after single Xe flashes) in the presence of agents that increase membrane conductivity. 26 μM bacteriochlorophyll, 50 μM PMS, pH 7 7, in all cases Each point represents the average of 10 measurements ●, valinomycin, 0 1 M potassium phosphate buffer ○, valinomycin, 0.1 M sodium phosphate ▲, CCCP, 0.1 M Tris–HCl.

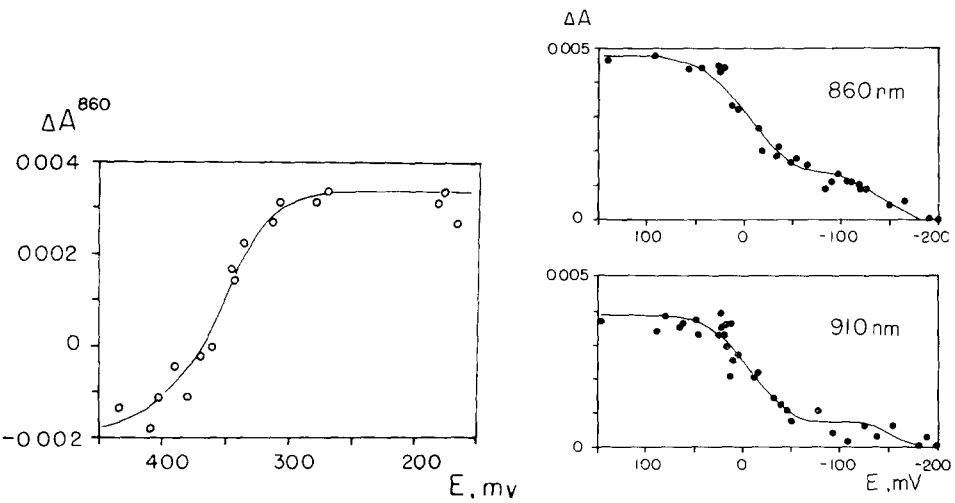

Fig. 5. Absorbance changes at 860 nm caused by single, saturating laser flashes, as a function of the redox potential, E, from 150 to 450 mV 40 μM bacteriochlorophyll, 50 μM PMS, 0 1 M potassium phosphate, pH 7 7, 0 4 M sucrose. The points are fit to a theoretical $2C-C^2$ curve, in which C follows a theoretical one-electron titration with E_m = 333 mV (see ref 21).

Fig. 6. Absorbance changes at 860 and 910 nm caused by single laser flashes, as a function of the redox potential from −200 to 150 mV. 40 μM bacteriochlorophyll, 0 1 M potassium phosphate, pH 7.7, 0.4 M sucrose, 50 μM PMS, 100 μM indigodisulfonate, 100 μM indigotetrasulfonate In both parts of the figure, the points are fit to theoretical one-electron titration curves in two steps with E_m = −3 mV and −140 mV

to reduce P_{870}^+ immediately after the flash, the lower parts of the figures show the well-known difference spectrum due to P_{870} photooxidation (see *e.g.* ref. 25). When cytochrome *c*-555 photooxidation was prevented in this way, no bacteriochlorophyll band shift could be detected (Fig. 3). A carotenoid shift still could be seen at high redox potentials, but its amplitude was only about one third of that which occurred at lower potentials (Fig. 2).

Figs 5 and 6 display in greater detail the dependence of the bacteriochlorophyll band shift on the redox potential. As one raises the potential above +300 mV, the band shift diminishes in amplitude (Fig. 5). The titration curve is indistinguishable from the titration[21] of cytochrome *c*-555 photooxidation on a single flash. Because each photosynthetic reaction center contains two cytochrome *c*-555 hemes, the titration does not follow a standard Nernst curve. Instead, the apparent midpoint is about 20 mV more positive than the true E_m of the cytochrome[21].

The bacteriochlorophyll band shift also decreases as one lowers the potential through 0 mV (Fig. 6). Titrating through this region reduces a second cytochrome, *c*-552, and the photooxidation of cytochrome *c*-552 replaces that of cytochrome *c*-555[18-21,26]. A small bacteriochlorophyll shift persists at lower potentials, but disappears completely as E goes below −200 mV. The midpoint potential of this final step is approximately −140 mV, which is the E_m of X, the primary electron acceptor for P_{870}[21].

Fig. 7 shows redox titrations of the changeover from the photooxidation of cytochrome *c*-555 to that of cytochrome *c*-552, following each of two, closely spaced flashes. The two curves are separated by about 40 mV; they lie on either side of a chemical (dark) titration curve of cytochrome *c*-552, which has an E_m of approximately 8 mV[21]. The titration curve for the first flash has a midpoint of approximately 20 mV, significantly above the E_m of −3 mV for the first step of the bacteriochlorophyll band shift titration (Fig. 6). Under Discussion, we shall show that this difference is consistent with the conclusion that the bacteriochlorophyll band shift accompanies the photooxidation of cytochrome *c*-555.

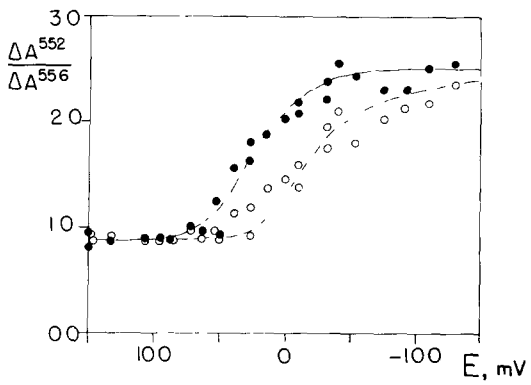

Fig 7 Redox titration of the changeover from the photooxidation of cytochrome *c*-555 (at high E) to that of cytochrome *c*-552 (at low E). ●, ratio of absorbance change at 552 nm to that at 556 nm, following a single laser flash ○, same, but following a second flash, 10 ms after the first Conditions essentially as in Fig 6 See refs 18 and 20 for spectra of the two cytochromes

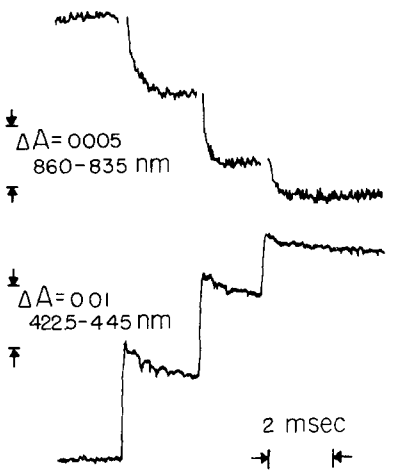

Fig. 8 Bacteriochlorophyll band shift and cytochrome *c*-555 oxidation, on three saturating flashes spaced approximately 2 ms apart Q-switched lasers provided the first and third flashes, a Xe lamp, the second. Approximately 30 μM bacteriochlorophyll, 100 μM PMS, 0 4 M sucrose, 1 0 M Tris–HCl, pH 7 7 $E = 247 \pm 22$ mV. To minimize the contribution of absorbance changes due to P_{870} photooxidation, the upper trace shows the difference between the flash-induced absorbance changes at 860 nm and those at 835 nm The lower trace shows the difference for the wave-length pair 422 5 and 440 nm. The differences were computed from the averages of 10 measurements at each wavelength. The gaps in the upper trace represent fluorescence artifacts at the time of the flashes The kinetics in this trace are instrument-limited and do not indicate the rate of the band shift.

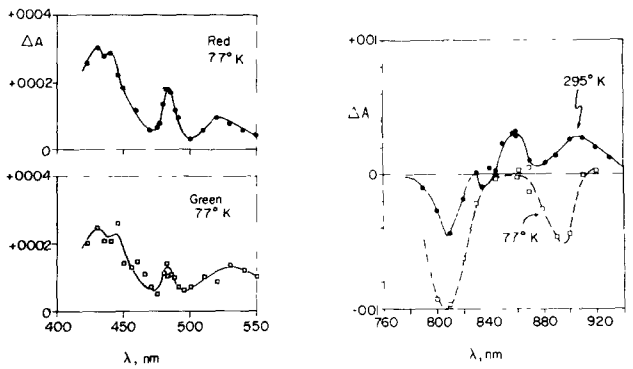

Fig. 9. Left Flash-induced visible absorbance changes at 77 K Approximately 115 μM bacteriochlorophyll, 50 μM PMS, 0.2 M choline–HCl, 0 01 M Tris–morpholinopropanesulfate, pH 7 7, 50 % glycerol For all samples, ΔA at room temperature was −0 009 at 422 nm, both before freezing and after freezing and rewarming Light path, 0 16 cm Before freezing, E under conditions similar to these ranged between 100 mV and 250 mV. Red, normal chromatophores Green, chromatophores prepared from cells grown in the presence of 30 μM diphenylamine. Right. Flash-induced infra-red absorbance changes at 295 °K (●) and at 77 °K (□). Normal chromatophores, approximately 155 μM bacteriochlorophyll Conditions as for the left part of the figure. $\Delta A_{422\ nm}$ was the same after freezing and rewarming as it was before freezing The signal from a photodiode was fed through an AC-coupled amplifier to an oscilloscope. The rise time constant of the amplifier was 0 8 ms, the decay, 100 ms. At room temperature, cytochrome *c*-555 reduces P_{870}^+ with a half-time of 2 μs, so absorbance changes due to P_{870}^+ were not detected

Fig. 8 compares the absorbance changes of the bacteriochlorophyll band shift with those due to cytochrome *c*-555 photooxidation, following each of three closely spaced flashes. The redox potential was 240 mV, poising cytochrome *c*-555 in the reduced form and cytochrome *c*-552 in the oxidized form. The amounts of cytochrome oxidation on the three flashes fall in the ratio 1.0 : 0.87 : 0.50. The absorbance changes due to the bacteriochlorophyll band shift follow essentially the same ratio (1.0 : 0.89 : 0.49). In this experiment, the amount of cytochrome photooxidation that occurs on the third flash depends on the amount of cytochrome reduction that occurs between the flashes, as well as on other factors; *cf.* ref. 21.

Fig. 9 shows absorbance changes that follow the illumination of chromatophores at 77 °K. At this temperature, photooxidation of P_{870} and *c*-552 can occur, but there is little or no photooxidation of *c*-555[26]. *E* was poised before the samples were cooled so as to provide P_{870} in the reduced state, and cytochrome *c*-552 in the oxidized state. The spectra therefore show an absorbance increase at 430 nm due to P_{870} photooxidation[27,28]. Although an absorbance increase also occurs at 482 nm (see also ref. 28), a similar absorbance change occurs in chromatophores from cells that have been grown in the presence of 30 μM diphenylamine (Fig. 9, left) Among other effects, diphenylamine strongly inhibits biosynthesis of carotenoids[29]. Cells grown in its presence are green rather than red, and typical carotenoid peaks are absent from the absorption spectrum. Illumination of the green chromatophores at room temperature gave no indication of a carotenoid band shift (*i.e* $\Delta A_{482\text{ nm}}$ *minus* $\Delta A_{491\text{ nm}}$ was zero within experimental error). We conclude that the 482 nm absorbance increase at 77 °K is not due to a carotenoid band shift. Thus, the carotenoid band shift seems not to occur if cytochrome *c*-555 photooxidation is blocked by lowering the temperature.

The right side of Fig. 9 compares spectra of the near infrared absorbance changes that follow illumination of normal (red) chromatophores at 295 K and 77 °K. The light-induced difference spectrum at 77 °K resembles that of P_{870} photooxidation at room temperature (*cf.* Fig. 3), except for an increase in the magnitude

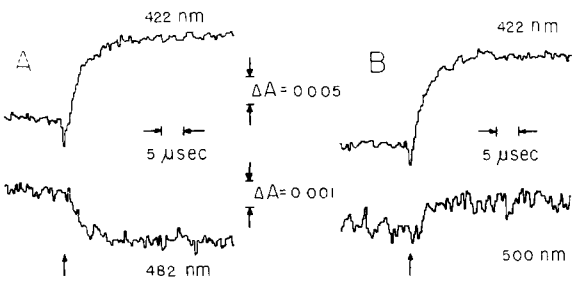

Fig 10. Rise kinetics of the carotenoid band shift, following single laser flashes, as measured at 482 nm (Expt A) and at 500 nm (Expt B), compared with the cytochrome *c*-555 photooxidation kinetics, as measured at 422 nm with the same suspensions of chromatophores Conditions as in Fig 8. The initial absorbance increases in the measurements at 422 nm are due to P_{870} oxidation. They serve as markers for the time of the actinic flash (arrows) Each trace is an average, computed from 25 separate measurements at 422 nm, 50 at 482 nm, or 100 at 500 nm The individual measurements were made approximately 3 min apart Measurements at slower sweep rates showed absorbance changes of the same size as the fast changes shown in this figure This comparison is important because only part of the absorbance change at 482 or 500 nm is due to the carotenoid band shift (see Fig 1D).

of the absorbance change around 800 nm. Apparently, the bacteriochlorophyll band shift also does not occur if cytochrome *c*-555 photooxidation is blocked by lowering the temperature.

Fig. 10 shows the rise kinetics of the carotenoid band shift, along with the kinetics of cytochrome *c*-555 oxidation in the same samples. The absorbance changes at 482 and 500 nm appear to occur simultaneously with the cytochrome oxidation Because the cytochrome itself contributes about 40 % of the absorbance changes at these wavelengths (Fig. 1D), and because the signal-to-noise ratio in the measurements is low, one must interpret the measurements with caution. The results are consistent with the conclusion that the carotenoid band shift and the cytochrome oxidation occur with the same kinetics, but it is difficult to rule out the possibility that a small portion of the shift occurs at a higher rate. Fluorescence artifacts prevented us from making similar measurements of the bacteriochlorophyll band shift.

DISCUSSION

The sensitivity of the bacteriochlorophyll band shifts to agents that specifically increase the electrical conductivity of the chromatophore membrane strongly suggests that the absorbance changes reflect a physical phenomenon, rather than an oxidation or reduction of the bacteriochlorophyll itself. The observation that exogenous oxidants or reductants do not affect the rate of decay of the band shifts after a flash supports this conclusion. The absorbance changes at 810, 836, 850, and 905 nm all behave alike in these respects so there is no basis for associating any of these wavelengths directly with a specialized, reactive bacteriochlorophyll complex.

Because the bacteriochlorophyll band shifts do not occur at potentials more positive than the E_m of P_{870} (480 mV[21]), nor at potentials much below the E_m of X, there is no reason to postulate the involvement of any photochemical reaction center other than the P_{870}–X system. Instead, the titration curves suggest simply that the band shifts require the photooxidation of cytochrome *c*-555 by P_{870}^+ The high-potential titrations (Fig 5) obviously agree with this conclusion, but the low-potential titrations (Figs 6 and 7) require further discussion.

High-potential titrations of cytochrome *c*-555 photooxidation following two flashes[21] are similar to the changeover titrations of Fig. 7. In both cases, the titration curves for the first and second flashes lie about 20 mV on either side of the chemical titration. Our interpretation of Fig. 7 therefore parallels the one that we have offered[21] for the high-potential titrations Assume that each photochemical reaction center contains two cytochrome *c*-552 hemes and two cytochrome *c*-555 hemes, and that any of the four hemes can donate an electron to P_{870}^+. Assume further that electron transfer between the two types of cytochromes allows the system to approach thermodynamic equilibrium shortly after the flash. Because cytochrome *c*-552 has the lower E_m, photooxidation of this cytochrome will prevail at equilibrium after the first flash if either of the two *c*-552 hemes is reduced prior to the flash Oxidation of cytochrome *c*-552 by the second flash, however, requires that both *c*-552 hemes be reduced initially The potential at which half of the photosynthetic units have at least one of the *c*-552 hemes initially in the reduced form is about 20 mV above the E_m of the cytochrome, and the potential at which half of the units have both *c*-552 hemes reduced is about 20 mV below the E_m.

We can now account for the bacteriochlorophyll shift titration in Fig. 6. Assume that the bacteriochlorophyll shift occurs only if P_{870}^+ initially oxidizes cytochrome *c*-555, but that the shift remains after the flash, even if the cytochrome *c*-555 is reduced rapidly by cytochrome *c*-552. That the bacteriochlorophyll shift can remain after cytochrome *c*-555 is reduced by other electron donors is clear from Fig. 1. Because the rate constant for the oxidation of cytochrome *c*-552 by P_{870}^+ is about twice that for the oxidation of cytochrome *c*-555[18-20], the former reaction path must predominate at low redox potentials. However, about 1/3 of the initial reaction could involve cytochrome *c*-555, even at very low redox potentials. This would account for the occurrence of the bacteriochlorophyll shift with reduced amplitude in the region between − 140 and 0 mV (Fig. 6). The changeover of the initial reaction path, from complete oxidation of *c*-555 at high potentials to 1/3 oxidation of *c*-555 at low potentials, would follow the chemical titration of *c*-552. The E_m of the bacteriochlorophyll band shift titration (Fig. 6) therefore should be about 20 mV below the midpoint of the left-hand titration in Fig. 7, as it is.

Light-induced carotenoid band shifts have been observed in *Rps. spheroides*, *Rps. gelatinosa* and *Rhodospirillum rubrum* with half-times of less than 1 μs[11,15,30]. The very fast kinetics and the dependence of these shifts on temperature and *E*[26,28,31,32] suggest a direct link to the photooxidation of P_{870}, rather than to cytochrome oxidation. Recently, Dutton and Jackson[33-35] have observed additional slower increments of the carotenoid band shift in *Rps. spheroides*. The slower phases correspond kinetically and thermodynamically to the photooxidation of two different cytochromes. Potentiometric studies suggest that the occurrence of cytochrome photooxidation also can increase the light-induced carotenoid shift at 77 °K in *Rps. gelatinosa* chromatophores[26]. The correlation between the band shifts and specific electron transfer reactions thus appears to be a common phenomenon in bacterial photosynthesis, although the contribution of each electron transfer reaction may vary from species to species.

The finding that the light-induced band shifts in *C. vinosum* accompany the photooxidation of cytochrome *c*-555, but not that of P_{870} or cytochrome *c*-552, seems to us to be a serious obstacle to attempts to interpret the band shifts simply in terms of a uniform transmembrane electrical field. The E_m values of P_{870} and cytochrome *c*-555 depend almost identically on the ionic strength of the solution, indicating that the two photooxidations cause very similar changes in the net electrical charge on the chromatophore membrane[36]. Neither oxidation results in the transport of an ion across the membrane[36]. If the band shift is an electrochromic effect, the bacteriochlorophyll and carotenoids would appear to sense an electrical field that depends uniquely on cytochrome *c*-555.

On the other hand, theories that rest on a local electric field due to the cytochrome itself (*e.g.* ref. 17) seem inconsistent with the persistence of the band shifts after cytochrome *c*-555 regains an electron (Fig. 1). Such theories also have difficulty accounting for the spectra of Fig. 3. These spectra and the earlier measurements of Vredenberg and Amesz[7] show that the bacteriochlorophyll shift encompasses the bulk chlorophyll absorption bands at 800 (or 820) and 850 nm, as well as that at 890 nm. The absorption spectra of subchromatophore particles prepared with detergents suggest that only the 890-nm bacteriochlorophyll and a small part of the 800-nm bacteriochlorophyll reside on the bacteriochlorophyll–protein complex that houses

P_{870} and the cytochromes[37]. If this is correct, the cytochrome photooxidation would appear to trigger a response of the membrane as a whole.

We conclude that neither a uniform transmembrane electrical field, nor a local electrical field provides a completely satisfying explanation of the light-induced band shifts

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