THE LACK OF CAROTENOID BAND SHIFTS IN A NON-PHOTOSYNTHETIC, REACTION CENTERLESS MUTANT OF *RHODOPSEUDOMONAS SPHEROIDES*

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Received 20 December 1971

1. Introduction

Recent work has shown that in photosynthetic tissues changes in the absorption spectrum of carotenoids can be correlated with the high energy state that mediates phosphorylation [1–6]. The carotenoid band shift can be produced by light, by KCl in the presence of valinomycin [6], and by pyrophosphate [3, 4]. We have sought to measure these effects in a non-photosynthetic mutant strain of *Rhodopseudomonas spheroides*. This mutant strain, PM-8, was obtained by Sistrom [7] and has been shown to lack reaction centers P870 and all manifestations of the primary photochemistry [7–9]. PM-8 was derived from the photosynthetically competent strain Ga, a spontaneous mutant of wild strain 2.4.1 (van Niel). Both PM-8 and Ga contain light-harvesting bacteriochlorophyll (BChl) and just two carotenoids, neurosporene and hydroxy-neurosporene [7]. When grown in the dark under the same conditions of aeration, strains PM-8 and Ga develop similar concentrations of BChl and carotenoids. Our measurements were made with both strains grown aerobically in darkness, and also Ga grown photosynthetically. We will show that the carotenoid band shifts can be produced by all the foregoing methods in aerobically grown cells of Ga, but none can be induced in strain PM-8. We will also show that this is not due to "leaky" membranes in chromatophores of PM-8, but that the presence of the reaction centers is necessary for the changes in the carotenoid spectra.

2. Materials and methods

Cells were grown according to Sistrom and Clayton [7], and chromatophores were prepared as described by Worden and Sistrom [10], except that light chromatophores were obtained using a discontinuous sucrose gradient containing 34.5% and 42.8% sucrose. Optical absorption changes were measured with a homemade device that has been been described previously [11]. The KCl + valinomycin and pyrophosphate experiments were carried out according to Jackson and Crofts [6], and Baltscheffsky [3, 4], respectively. The partition of H^+ inside and outside the chromatophores was monitored by the fluorescence of atebrin, according to the techniques of Avron [12, 13]. The fluorescence was measured with an RCA 1P28 photomultiplier, exciting at 400–440 nm and measuring at 460–600 nm. The K^+-specific electrodes were type MI-330 obtained from Microelectrodes, Inc. by Dr. Roger Spanswick at Cornell. They were filled with potassium liquid exchanger (Corning No. 477317) and assembled by the method of Walker [14]. The electrode was connected to a Corning Model 10 pH meter and the output was monitored on a Bausch and Lomb VOM-5 recorder. Valinomycin was obtained from Sigma, while X-464 and Gramicidin were donated by Dr. A. Jagendorf.

3. Results

3.1. *Strain Ga*

The absorption changes in the carotenoid spectrum of *R. Spheroides* Ga chromatophores that are induced

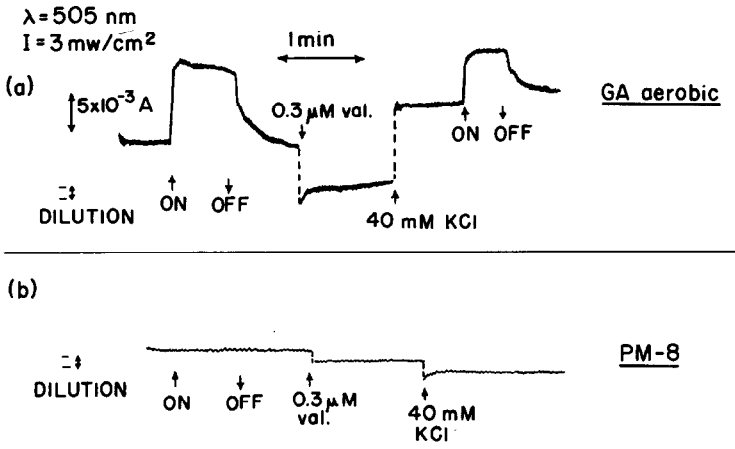

Fig. 1. Carotenoid adsorption changes induced by light, valinomycin, and KCl. The cuvette of 1 cm path length contained chromatophores at a concentration of 8 μg BChl/ml [18] suspended in 1.0 mM MES, pH 6.5 + 100 mM choline chloride. The final concentrations of the substances added are as indicated. Actinic light was supplied by a Sylvania "sun gun", 650 W, filtered through an 800 nm Baird-Atomic interference filter. In all of the experiments both Ga and PM-8 chromatophores had a BChl to carotene ratio of 2.0–2.5 [8].

by light, valinomycin, and KCl are shown in fig. 1(a). Illumination produced a rapid absorption change which decayed slightly during continuous illumination, and then returned to the initial level in the dark following cessation of illumination. The addition of valinomycin either by injection or mixing caused an absorption change in the opposite direction to that induced by light. Further addition of 40 mM KCl produced an absorption change which was now in the same direction as the light-induced change. Both of these absorption changes were much larger than the trivial change caused by comparable dilution of the chromatophore suspension, as determined by injection of water, choline, 0.25 M sucrose and NaCl in the presence of valinomycin or KCl in the absence of valinomycin. All of these effects in chromatophores from aerobically grown cells were, in all qualitative respects, similar to those found in photosynthetically grown *R. Spheroides* wild type [6].

When those absorption changes were measured every 5 nm throughout the carotenoid region, the spectrum seen in figs. 2–5 were obtained. The light induced spectrum for chromatophores from photosynthetically grown Ga is shown in fig. 2(a). Typically, this spectrum has peaks at 445, 475, and 505 nm, and troughs at 460 and 490 nm. The spectrum induced by 50 mM KCl in the presence of 0.3 μM valinomycin is shown in fig. 2(b). This spectrum is qualitatively identical to that produced by light. Similar results were obtained from chromatophores of *R. Spheroides* Ga grown aerobically, with slight changes in the form of the spectrum as shown in fig. 3. The altered shape is seen in the KCl-induced spectrum as well as the one induced by light. In agreement with Jackson and Crofts [6], the magnitude of the absorption change varied with the logarithm of the KCl concentration in these preparations (unpublished observations).

Shifts in the carotenoid spectrum could also be induced by the addition of HCl and KOH in the presence of CCCP [6]. These changes could be produced in the absence of the uncoupler, but since CCCP renders the membrane permeable to protons [15, 16], the magnitude of the changes could be increased 2–3-fold in its presence. The absorption changes were in the same direction (for HCl) or in the opposite direction (for KOH) as the light-induced changes. The KOH-induced change and that induced by valinomycin were both mirror images of the light-induced spectra. These results imply that the light-, KCl- and HCl-induced absorption changes are produced by a potential which is positive inside. This is in agreement with results obtained on chromatophores from photosynthetically grown cells [6].

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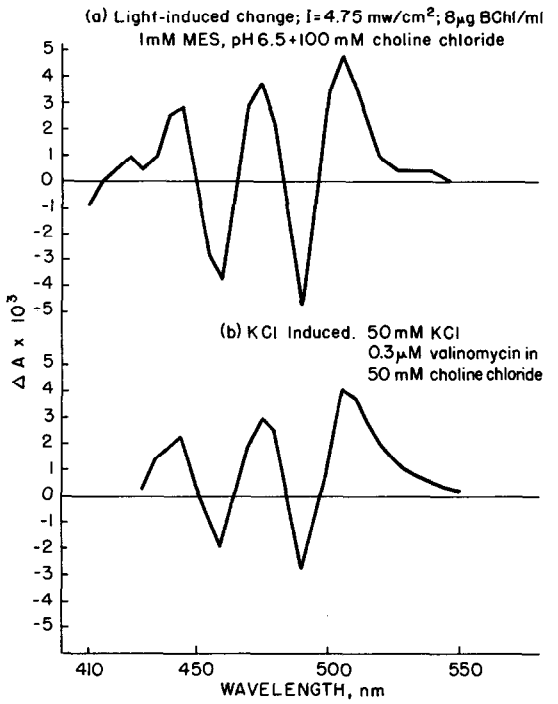

Fig. 2. Spectra of the carotenoid absorption changes in photosynthetically grown *R. Spheroides* Ga induced by light and by KCl in the presence of valinomycin. Conditions were as shown in fig. 1 and measurements were performed every 5 nm.

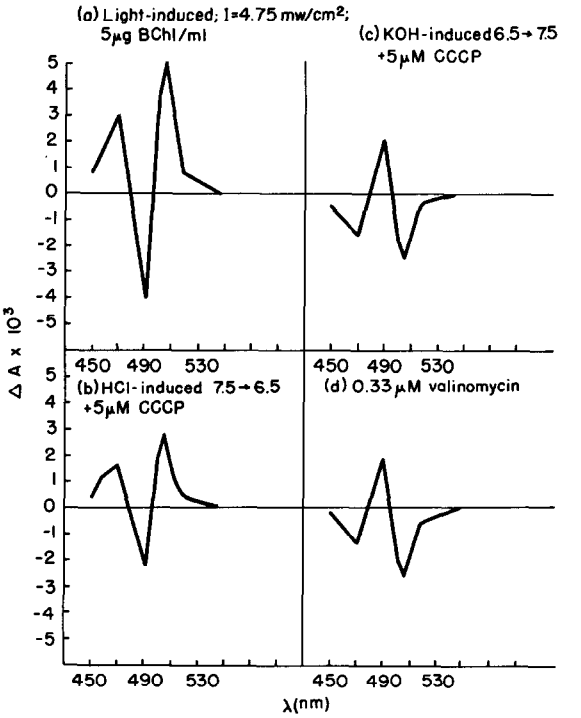

Fig. 4. Spectra of carotenoid absorption changes in aerobically grown *R. Spheroides* Ga induced by HCl and KOH in the presence of CCCP and by valinomycin. The chromatophores were suspended in 100 mM KCl and sufficient HCl or KOH was injected to produce a change of 1 pH unit.

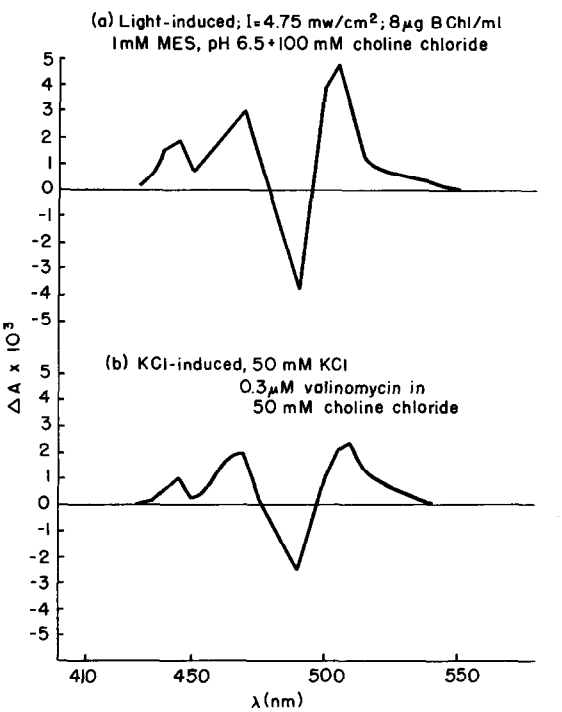

Fig. 3. Spectra of the carotenoid absorption changes in aerobically grown *R. Spheroides* Ga. Experimental conditions are the same as in fig. 2.

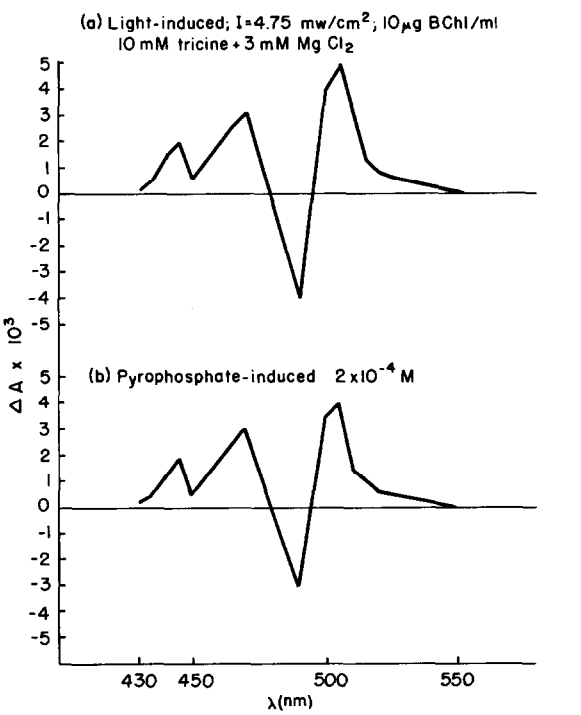

Fig. 5. Carotenoid band shifts in chromatophores of aerobically grown *R. Spehroides* Ga induced by light and pyrophosphate. Experiments were performed as in figs. 1 and 2.

method of inducing carotenoid changes in aerobically grown *R. Spheroides* Ga, we tried pyrophosphate as described by Baltscheffsky [3, 4]. When PP_i was injected into a suspension of light chromatophores from freshly grown cells, the spectrum seen in fig. 5(b) was obtained. Again, the pyrophosphate induced spectrum is equivalent in all respects to that induced by light (fig. 5(a)).

3.2. *Strain PM-8*

When valinomycin and KCl were added to chromatophores of the non-photosynthetic mutant PM-8, the results seen in fig. 1(b) were obtained. Neither valinomycin nor KCl caused absorption changes that were greater than that attributable to dilution. Similar negative results were obtained when absorption changes were monitored after the addition of pyrophosphate in the presence of $MgCl_2$, or for HCl and NaOH in the presence of CCCP. Different types of chromatophores, various concentrations of valinomycin, KCl, pyrophosphate, or CCCP did not produce any detectable carotenoid band shift. When measurements were performed at the highest sensitivity available, it could be determined that any existing absorption change had to be less than 3% of that obtained in the case of aerobically grown Ga. Therefore, we must conclude that carotenoid band shifts induced by light, KCl + valinomycin, or pyrophosphate do not occur in the non-photosynthetic mutant of *R. Spheroides.*

3.3. *Ion retention and permeation*

Two obvious answers readily explain these experimental results. First, the reaction centers may be directly involved in the production of the carotenoid band shift. Secondly, it is possible that since the reaction center protein is 20% or more of the total chromatophore protein [9], the absence of this protein does not allow formation of "intact" chromatophores. In other words, the PM-8 chromatophores could be "leaky" to small ionic species like K^+, and therefore unable to maintain a membrane potential.

Two experiments were devised to test this latter hypothesis. In the first, chromatophores were exposed to a pH shift in the presence of atebrin. The redistribution of H^+ across the membrane was assumed to be indicated by the change in atebrin fluorescence. In the second, the efflux of K^+ in the dark after addition of ionophorous antibiotics was measured directly by use of a K^+ selective electrode. Both methods gave the same results – the chromatophores of PM-8, like those of Ga, have semi-permeable membranes and are capable of forming diffusion gradients and membrane potentials.

The experiment with atebrin depends on the assumptions that only the uncharged species of atebrin can move through the membrane [13], and that atebrin is distributed across the membrane in the same ratio as the proton concentration [12, 13]. The fluorescence of atebrin is quenched when the substance is taken up by chromatophores or chloroplasts [12, 13, 17].

The presence of intact chromatophore membranes is expected to influence the fluorescence of atebrin in two ways: by holding some of the atebrin in the inner phase (in proportion to the H^+ concentration), and by altering the fraction of the total atebrin that is protonated, at any given external pH. Both effects should make the fluorescence less responsive to changes of external pH. The second of the two effects could be eliminated by making the measurements at pH values distant from the pK of atebrin (7.6), but this would entail formidable technical difficulties. In any event, both effects are symptomatic of the membranes to sequester protons in an inner phase. The point to be emphasized is that chromatophores of PM-8 behave like those of Ga, and not like sonicated chromatophores.

The changes in atebrin fluorescence that were induced by a pH jump can be seen in table 1. In the absence of chromatophores, the fluorescence of atebrin rose almost 2.5 times when the pH of the solution was changed from 7.1 to 8.6. When either Ga or PM-8 chromatophores were present, the atebrin fluorescence rose only half as much, implying that some of the atebrin has been taken up by the chromatophores, causing a relative quenching of fluorescence. Corresponding results were obtained for the reverse pH jump. When the Ga chromatophores were extensively sonicated, pH jumps yielded changes that were no different from those with atebrin alone, implying, that intact vesicles are necessary to produce the observed changes. In all cases, PM-8 acted very much like Ga.

When valinomycin was added to chromatophores of Ga and PM-8 in the dark, and the external K^+ concentration was monitored, the results seen in fig. 6

Table 1
pH-Induced change in atebrin fluorescence.

pH 8.2 ⟶ 7.1		pH 7.1 ⟶ 8.6	
Addition	Fluorescence (arbitrary units)	Addition	Fluorescence (arbitrary units)
All samples; pH 8.2	100	All samples; pH 7.1	100
Atebrin; pH 7.1	54	Atebrin; pH 8.6	240
Atebrin + Ga; pH 7.1	72	Atebrin + Ga; pH 8.6	153
Atebrin + PM-8; pH 7.1	70 (Atebrin	Atebrin + PM-8; pH 8.6	176 (Atebrin
Atebrin + Ga (sonicated); pH 7.1	56 efflux)	Atebrin + Ga (conicated); pH 8.6	235 uptake)

Atebrin fluorescence was excited by weak light through a Baird-Atomic 420 nm interference filter of band width 40 nm. Emmission was measured with an 1P-28 photmultiplier through C.S. 3-71 and C.S. 4.96 Corning filters. The atebrin concentration was 3 μM, though identical results were obtained between 0.3–3.0 M. Sonication was carried out on a Bronson sonifier at 75% of full output for 2 min. pH changes were made using NaOH or HCl and were measured on a Beckman Zeromatic II using a Thomas Combination electrode. All samples were normalized to 100 at the starting pH. Chromatophores were at a concentration of 4 μg BChl/ml.

Table 2
Potassium efflux in the presence of ionophorous antibiotics.

	GA		*PM-8*	
Addition	External K^+ (mM)	K^+ effluxed (mM)	External K^+ (mM)	K^+ effluxed (mM)
10 mM MES; pH 7.1	1.0	3.5	1.0	2.0
+0.5 μM valinomycin	4.5		3.0	
10 mM MES; pH 7.1 +0.1 mM KCL	1.2	1.5	1.2	1.1
+0.2 μM X-464 (Nigericin)	2.7		2.3	
10 mM MES; pH 7.1 +0.1 mM KCL	1.2	3.3	1.2	1.3
+0.5 μM valinomycin	4.5		2.5	
10 mM MES; pH 7.1 +0.1 mM KCL	1.2	1.7	1.2	1.2
+1.5 μM gramicidin	2.9		2.4	

The K^+ electrode was standarized against KCl solutions of known concentration. The output was found to be proportional to the log $[K^+]$ in the range of 1–100 mM. The experiments were performed as described in fig. 6.

were obtained. In both cases, an efflux of K^+ could be seen immediately after addition of the antibiotic. When the electrode was standardized against solutions of known K^+ concentrations, the results could be tabulated in concentrations as shown in table 2. The addition of 0.5 μM valinomycin to PM-8 chromatophores caused the efflux of a substantial amount of K^+. We must conclude, therefore, that the membranes of PM-8 chromatophores can conserve ion concentrations nearly as well as those from Ga. The PM-8 chromatophores should therefore be able to sustain membrane potentials comparable to those in Ga.

4. Discussion

We have shown that chromatophores from the photosynthetically competent strain Ga of *R. Spheroides* exhibit carotenoid band shifts, induced either by light or by chemical perturbations designed to alter the membrane potential. The band shifts have been shown in the past [2, 5] to depend on the inactness of the chromatophore membranes that can, in principle, conserve ion gradients. These shifts are seen in preparations from cells grown aerobically in

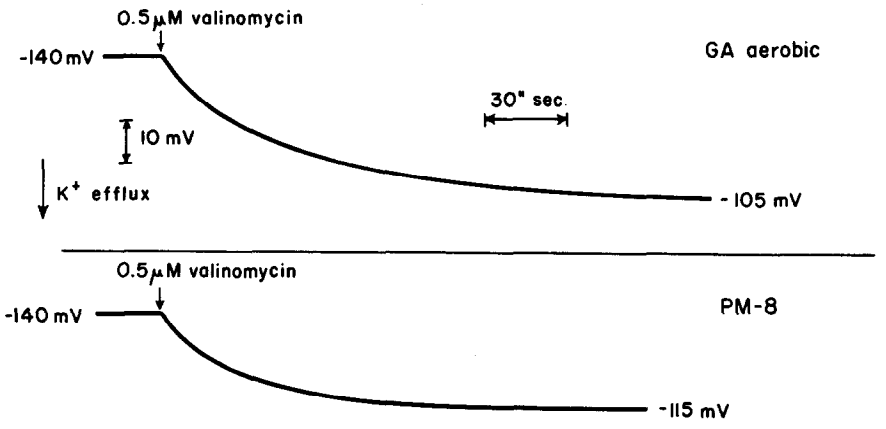

Fig. 6. Efflux of K^+ in *R. Spheroides* chromatophores induced by valinomycin in the dark. Chromatophores containing 20 μg BChl from Ga or PM-8 were suspended in 10 mM MES, pH 7.1 to a final vol of 4 ml. Valinomyin was added to a final conc. of 0.5 μM. The entire apparatus was shielded with a Faraday cage due to the high electrical resistance of the microelectrode.

the dark as well as those grown photosynthetically. Chromatophores from the non-photosynthetic mutant strain PM-8, endowed with normal concentrations of light harvesting BChl and carotenoids but lacking reaction centers, exhibit no carotenoid band shifts. The membranes of PM-8 chromatophores can sustain concentrations of K^+ and H^+, and should be capable of developing membrane potentials comparable to those in strain Ga. These results imply that the carotenoid shift is mediated in some specific way by the reaction centers, by a mechanism as yet unknown. It is possible that the lack of reaction centers changes the membrane organization enough to prevent the occurrence of the band shift. It is likely that, whatever the mechanism, only those caretenoids in close proximity to the reaction centers are involved in the band shift.

Acknowledgements

This investigation was supported by grant No. AT(30-1)-3759 from the Atomic Energy Commission. We would like to thank Dr. William Sistrom for suggesting some of these experiments.

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