THE HIGH ENERGY STATE IN CHROMATOPHORES FROM *RHODOPSEUDOMONAS SPHEROIDES*

J.B. JACKSON and A.R. CROFTS

Department of Biochemistry, University of Bristol, Medical School, Bristol, BS8 ITD, England

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1. Introduction

The equilibrium high-energy state under non-phosphorylating conditions in chromatophores from *Rhodospirillum rubrum* has been shown to be associated with a transmembrane pH gradient of about 1 unit [1] and a membrane potential in excess of 80 mV [1,2]. Similar results are obtained with chromatophores from *Rps. spheroides.* In chromatophores from both *R. rubrum* and *Rps. spheroides,* under conditions of energy conservation changes occur in the absorption spectrum which are associated with carotenoids [3–7]. In this paper we discuss the effects on the spectrum of carotenoids of *Rps. spheroides* of generating electrical potentials in the dark across the membranes of chromatophores by ionic gradients operating through ionophorous antibiotics and uncoupling agents [8–11]. We find that a shift in the carotenoid spectrum is induced which is similar to that observed in the light [7] when a potential is generated which is positive with respect to the inside of the chromatophores.

2. Results

The time course of changes in the carotenoid spectrum of chromatophores from *Rps. spheroides* initiated by illumination or by addition of ions in the presence and absence of ionophorous reagents is shown in fig. 1.

Abbreviations used: FCCP, carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone; BChl, bacteriochlorophyll; MES, 2(*N*-morpholino)ethane sulphonic acid.

Illumination induced a rapid change, the extent of which decayed during the subsequent illumination period, and which returned to the initial level in the dark [7]. The illumination response was not greatly influenced by the ionic constitution or pH of the suspending medium. Addition of valinomycin to chromatophores suspended in a choline chloride medium gave rise to a change in the opposite direction to that induced by light which slowly decayed. Subsequent addition of KCl gave rise to a change in the same direction as that induced by light (fig. 1). No similar change was induced by addition of a similar concentration of NaCl of of choline chloride, or by KCl in the absence of valinomycin.

Addition of KOH or HCl to chromatophores suspended in KCl or choline chloride gave rise to small changes in the opposite (for KOH) and the same (for HCl) direction as the light induced changes (fig. 2). On adding FCCP, further additions of KOH and HCl gave rise to changes which were 2 to 3 times as extensive as the changes in the absence of FCCP.

The spectra of these changes are shown in fig. 3. The spectrum of the change induced by addition of KCl in the presence of valinomycin, or of HCl in the presence of FCCP was similar to that induced by light. The spectral change induced by addition of valinomycin in a choline chloride medium, or by KOH in the presence of FCCP was a mirror image of the change induced by light.

In table 1, the initial kinetics of the changes induced by light, by a KCl pulse in the presence of valinomycin, and by KOH and HCl pulses in the presence of FCCP are compared. The rise time of the change induced by a pulse from a Q-switched ruby laser

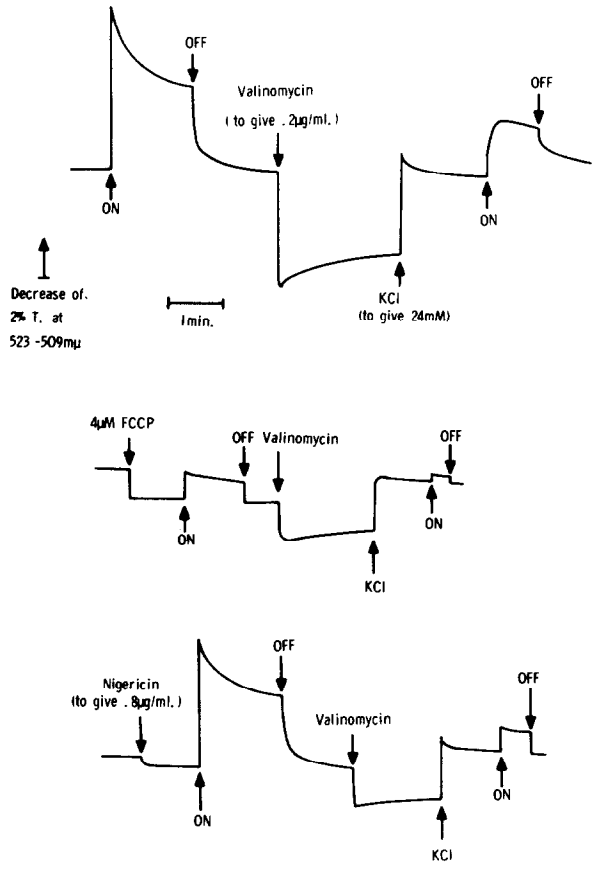

Fig. 1. Carotenoid changes induced by light and by K^+-gradients. *Rps. spheroides* cells were grown and chromatophores were prepared essentially as described previously for *R. rubrum* [1]. 10 µl of the stock chromatophore suspension, containing 0.015 mg. BChl [16], were suspended in 2.5 ml of 100 mM choline chloride, 20 mM MES pH 6.4 in a 1 X 1 cm clear-sided cuvette. Final concentrations of additions were as indicated. The transmission changes were measured on a recording double beam spectrophotometer at 523–509 mµ. Actinic illumination (Wratten 88A filter) was provided by a quartz-iodide lamp placed at right angles to the measuring beams. The photomultiplier was protected from the actinic light by a dilute copper sulphate filter.

was faster than 0.1 µsec. The rise times of the ion induced changes were very much slower, about 50–200 msec.

When the final concentration of KCl added as a pulse after addition of valinomycin was varied, the extent of the change in the carotenoid spectrum varied with the logarithm of the KCl concentration (fig. 4). Although the extent of the absorption change varied, the shift in the carotenoid spectrum was similar at low and at high final KCl concentration.

3. Discussion

The relation between carotenoid shifts in photosynthetic bacteria and their energetic state has been investigated in a number of laboratories [3–7]. In particular, Fleischman and Clayton [7] have suggested from the sensitivity of the changes observed in *Rps. spheroides* chromatophores to uncouplers, ionophores and electron flow inhibitors that "the phosphorylation intermediate is somehow intimately involved with the absorption band shift". They have considered direct interaction with electron carriers and the physical effect of a membrane potential or pH gradient on the pigment molecules as possible mechanisms for the carotenoid shift. In addition Baltscheffsky [6] has considered the possibility of conformational changes as a high energy state affecting the physical environment of pigment molecules and Amesz and Vredenberg [17] have suggested from the low quantum requirement for the shift in *Rps. spheroides* that the change follows from an alteration in the physical environment of the pigment molecules rather than by direct chemical reaction. Fleischman and Clayton [7] originally discounted the possiblity of effects due to a pH gradient because the slow kinetics of H^+-uptake [1,12] were not compatible with the rapid rise-time of the carotenoid change. However, our demonstration of the effects of ionophores on ion transport in *R. rubrum* chromatophores [12], and the recent work of Witt and his collaborators [13,14] suggested that the carotenoid shift may be a membrane potential indicator analogous to the 518 mµ change in chloroplasts [13].

In chomatophores the membrane is relatively impermeable to small ions [1], and the availability of reagents which specifically increase the permeability of the membrane to some ions, makes it possible to generate potentials across the membrane by ionic gradients.

Additions of valinomycin to *Rps. spheroides* chromatophores containing about 2 mM K^+ and suspended in choline chloride, leads to a loss of K^+ from the

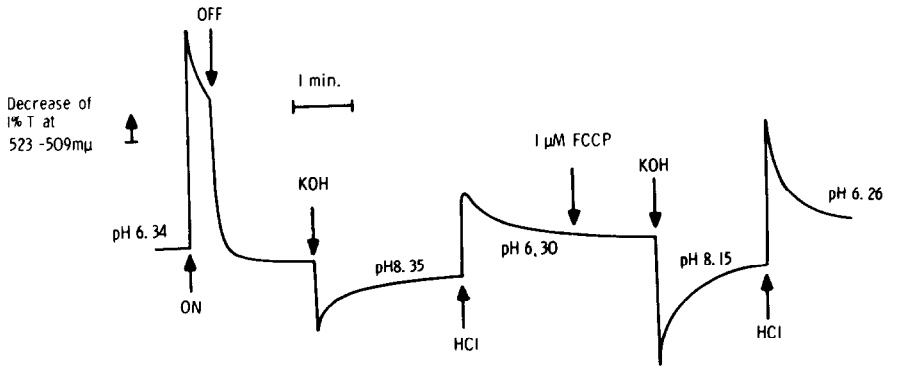

Fig. 2. Carotenoid changes induced by H^+ gradients. 20 μl of chromatophores were suspended in 6 ml of 100 mM KCl in a 2 X 1 cm cuvette (1 cm light path) accommodating a pH electrode and stirrer. The pH of the suspension during the experiment was as indicated. Additions of KOH and HCl were 0.5 μ eq. Otherwise as in fig. 1.

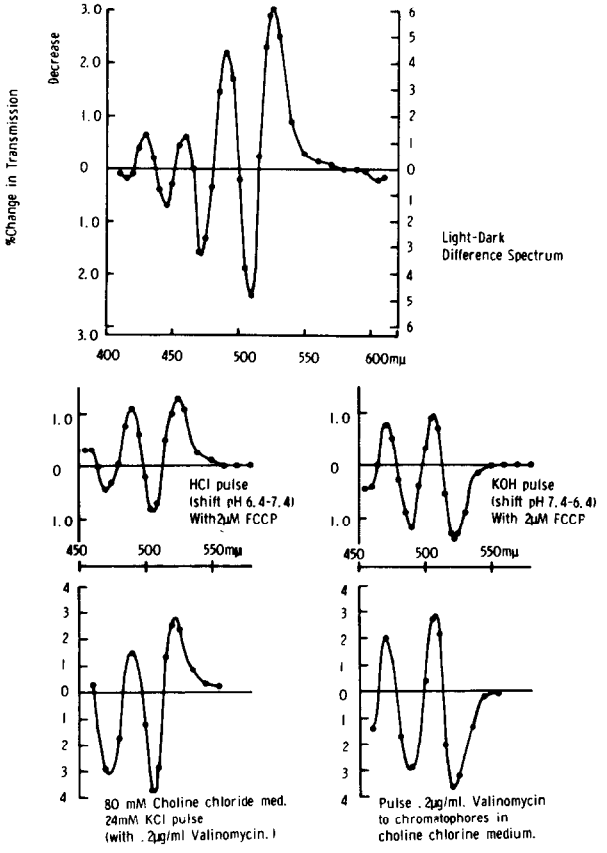

Fig. 3. Spectra of carotenoid changes induced by light and ion gradients. Each point was obtained from a series of experiments similar to those in figs. 1 and 2. The reference wavelength used was 587 mμ, which was shown to be isobestic in separate experiments with a single beam spectrophotometer.

chromatophores (see ref. [1]). The initial K^+-gradient on adding valinomycin would generate a potential negative with respect to the inside of the membrane, which would decay as equilibration of other ionic species led to dissipation of the potential and equilibration of K^+. After equilibration in the presence of valinomycin, the internal and external K^+ concentrations would be equal. Further addition of KCl to the medium would give rise to a gradient of K^+ which would generate a potential positive with respect to the inside of the membrane, and which would decay as other ions equilibrated. In each case, the extent and sign of the potential would vary with the gradient of chemical activity for K^+ according to the equation:

$$\Delta\psi_{R-L} = \frac{R\ T}{F} \ln \frac{(C^+)_L}{(C^+)_R} \simeq 60 \log_{10} \frac{(C^+)_L}{(C^+)_R}$$

where $\Delta\psi$ is the difference in potential in mV, and (C^+) is cation activity, which at the dilutions used is nearly equivalent to concentration.

This hypothetical series of events depends for its validity on the assumption, now well founded on experimental evidence, that valinomycin causes an increased membrane permeability specifically for K^+, Rb^+ and Cs^+ [8–10]. A similar argument can show that potentials would be generated across the chromatophore membrane by H^+-gradients operating through uncoupling agents such as FCCP which cause an increased membrane permeability specifically for H^+ [9,11].

Table 1

Agent causing carotenoid absorption change	Rise time (95%)	$t\frac{1}{2}$
Lase pulse	<0.1 μsec	–
HCl pulse (pH 7.7 → 5.9) in the presence of 1.25 μM FCCP	~1 sec	$\lesssim$200 msec
KCl pulse (giving 19 mM K^+) in the presence of 0.25 μg/ml valinomycin	~300 msec	$\lesssim$ 50 msec

Experiments were done using either a Q-switched ruby lase (0.67 mg BChl in 0.5 ml of 0.1 M KCl, 0.16 mm light path) or a pulse-flow, double-beam apparatus (523–509 mμ). We are grateful to Professor Britton Chance for enabling us to visit the Johnson Research Foundation, and for making these facilities available to us, and to Dr. W.W.Hildreth and Dr. D.DeVault for experimental assistance.

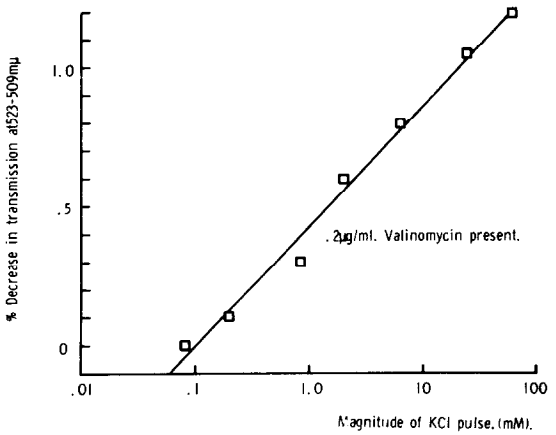

Fig. 4. Relation between the extent of the carotenoid change and the K^+-gradient. Experiments were performed as in fig. 1 (0.013 mg BChl). The size of the KCl pulse was varied and the final potassium concentration was as shown.

If carotenoids are indicators of membrane potential changes, a correlation between the spectral changes observed and the potential changes expected from the above arguments should be found. The changes shown in fig. 1 are consistent with the potential changes predicted from the ionic gradients induced, and the inhibitory effects of nigericin and FCCP on the carotenoid changes can be explained in terms of their effects on membrane permeability [1,9]. Of particular interest is the action of FCCP in enhancing the spectral changes induced by KOH or HCl pulses (fig. 2). The carotenoid changes are "energy-linked" [6,7] and such an enhancement would not be expected if FCCP acted by catalysing the hydrolysis of chemical intermediates responsible for the high-energy state. These results are therefore completely at variance with classical concepts of uncoupling action [15].

The correlation between the carotenoid changes and the expected membrane potential are more rigorously demonstrated by the results in fig. 4. The extent of the change (measured as the difference in absorption at a maximum and minimum of the difference spectrum) was proportional to the logarithm of the concentration of KCl added to the chromatophores. Since after equilibration with valinomycin the internal K^+ concentration was the same before each addition, the change was proportional to the logarithm of the K^+-gradient, as predicted from the equation. The slope of the graph gives the spectral change per decade of K^+-concentration difference, or per 60 mV from the equation. The intercept of the Y-axis gives the external K^+ concentration for null change, and this was found to be close to that of the external medium after equilibration of chromatophores with valinomycin in choline chloride. The consistency of the correlation over three decades of K^+-concentration suggests that the carotenoid change may provide a reliable measure of the electrical potential developed across the membrane.

The spectra of the changes induced by ionic gradients (fig. 3) were clearly related to the light induced spectral changes, being similar for gradients producing a positive potential inside, and "mirror-image" for gradients producing a negative potential inside.

The "mirror-image" spectral change can best be explained as a loss of a species giving rise to a spectrum of the light-induced type. The similarity between the light-induced carotenoid shift and that induced by ionic gradients provides strong evidence for the view that the light induced effect may also be in response to a membrane potential, which is equivalent to a high-energy state. If this is so, the potential generated in the light may be derived from the extent of the carotenoid change, by reading off from the slope of fig. 4. Typical values for chromatophores from the same suspension as that used for the experiments in fig. 4 are 430 mV for the maximal potential generated at the peak of the initial spike, and 190 mV for the potential during the steady state. This latter value is in close agreement with the value calculated from the K^+-gradient for null pH change on adding valinomycin to chromatophores in the light (J.B.Jackson and A.R.Crofts, unpublished results, see also refs. [1,2]), and gives a total steady-state proton motive force [11] of 240 mV when the co-existing pH gradient is allowed for.

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