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# ABSORPTION CHANGES OF CAROTENOIDS AND BACTERIOCHLOROPHYLL IN ENERGIZED CHROMATOPHORES OF *RHODOSPIRILLUM RUBRUM*

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## SUMMARY

The energization of *Rhodospirillum rubrum* chromatophores by the light, ATP, $PP_i$, by dark electron transfer *via* energy-coupling sites of the redox chain, by the combination of KCl and valinomycin causes absorption changes of carotenoids and bacteriochlorophyll. These changes due to the absorption-band shifts of the pigments are sensitive to the uncoupler *p*-trifluoromethoxycarbonyl cyanide phenylhydrazone (FCCP) but not to the combination of KCl and nigericin, which abolishes fluorescence changes of atebrin. Dithionite and ferricyanide depress the light-induced absorption changes of bacteriochlorophyll but have no inhibitory effect on the $PP_i$-induced changes. Analysis of bacteriochlorophyll absorption changes in the infra-red region shows that the photooxidation of bacteriochlorophyll reaction centers with the negative peak in the region of 890 nm is accompanied by red and blue shifts of bacteriochlorophyll absorption bands. These shifts are due to a transmembrane electrochemical gradient of $H^+$ and a local electric field arising as a result of oxidation of the reaction centers. It appears that the superposition of the (1) red shift which is characterized by negative and positive peaks at 865 and 895 nm, respectively, and (2) photobleaching of bacteriochlorophyll reaction centers in the region of 890 nm cause overall absorption changes with the negative peak at 865 nm.

## INTRODUCTION

According to Mitchell's chemiosmotic hypothesis[1], the redox chain and hydrolysis of ATP in coupling biomembranes can operate as "proton pumps" transporting $H^+$ against the concentration gradient of these ions. The investigation of the active transport of natural and synthetic penetrating ions has shown that the inner phase of *Rhodospirillum rubrum* chromatophores is charged positively, whereas the inner phase of cytoplasmic membrane is negatively charged in the energized state[2–5].

Abbreviations: FCCP, *p*-trifluoromethoxycarbonyl cyanide phenylhydrazone; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine

It was shown that the electrochemical potential generated on the membranes influences the state of the carotenoids in the *Rhodopseudomonas spheroides* chromatophores causing shifts in their absorption bands[6,7]. A similar effect was found for *R. rubrum* chromatophores[8,9] and chloroplasts[10].

The electric-field induced shifts of spectral lines was first shown in 1913 by Stark during his investigation of hydrogen luminescence. The term Stark's effect or electrochromic effect is used now for every action of the electric field on the absorption and luminescence spectra.

In the present study the action of the electric field generated in *R rubrum* chromatophores on the spectral characteristics of bacteriochlorophyll and carotenoids was investigated.

Preliminary reports of some of this work have been published[11,12]

## METHODS

The nonsulfur purple bacterium *R. rubrum* was grown and chromatophores were prepared as described previously[4].

The absorption changes in the *R. rubrum* chromatophores were measured with a single-beam difference spectrophotometer[13]. The actinic 597-nm light was obtained by means of glass-color and interference filters (the half band width was 8 nm). The intensity of the actinic light was usually about 9000 erg · $cm^2$ · s. Atebrin fluorescence was measured as described by Schuldiner *et al.*[15].

Experiments were performed on the aerobic suspensions of chromatophores. The incubation medium of chromatophores contained 0.25 M sucrose, 0.05 M Tris–HCl buffer (pH 7.6–7.8) and $5 \cdot 10^{-3}$ M $MgCl_2$. The other conditions are described in the figure captions.

## RESULTS AND DISCUSSION

Light-induced absorption changes with peaks at 790, 810, 865 and 895 nm were found in the aerobic suspension of *R rubrum* chromatophores in the presence of *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD) and ascorbate (Fig. 1, Curve 1).

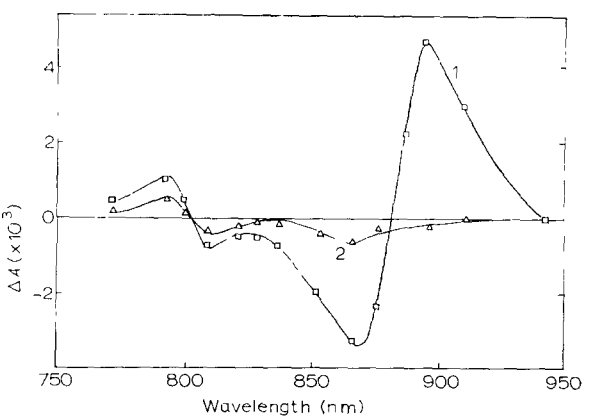



Fig 1. Absorption difference spectra (light *minus* dark) of *R rubrum* chromatophores in the presence of TMPD (0.1 mM) and ascorbate (1 mM) 1, without FCCP, 2, with 3 $\mu$M FCCP. $A_{880\ nm} = 3.3$

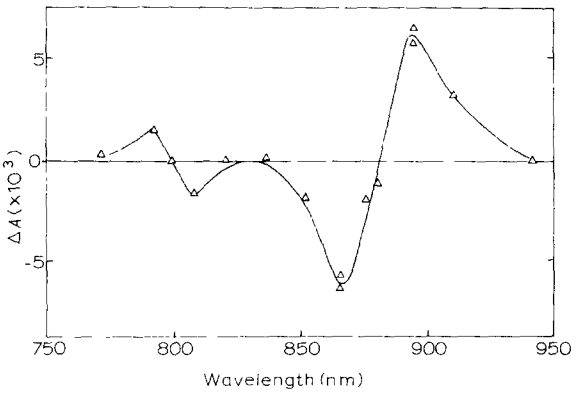



Fig 2 The spectrum of absorption changes induced by $PP_i$ (50 $\mu$M) in *R. rubrum* chromatophores in the dark, $A_{880\ nm} = 1\ 5$

These changes could be explained as a result of blue and red shifts of the bacteriochlorophyll absorption bands. The similar conclusion was previously made by Vredenberg and Amesz[16] for some bacteria.

An uncoupler of photophosphorylation, *p*-trifluoromethoxycarbonyl cyanide phenylhydrazone (FCCP) removes the red shift and reduces the amplitude of the difference changes linked with the blue shift (Fig. 1, Curve 2). It may well be that the spectral shifts of bacteriochlorophyll absorption bands are linked with the mechanism of energy coupling in the chromatophores.

This suggestion is supported by the data of Fig. 2 which shows the spectrum of bacteriochlorophyll absorption changes induced by the addition of $PP_i$ to the chromatophores suspension in the dark. $PP_i$ is seen to cause an effect similar to that induced by light in the presence of $TMPDH_2$.

Fig. 3A and B show the kinetics of $PP_i$-induced absorption changes of bacteriochlorophyll at 895 nm. The effect of $PP_i$ is prevented by the uncoupler FCCP.

The effect similar to $PP_i$ is also caused by hydrolysis of ATP and aerobic

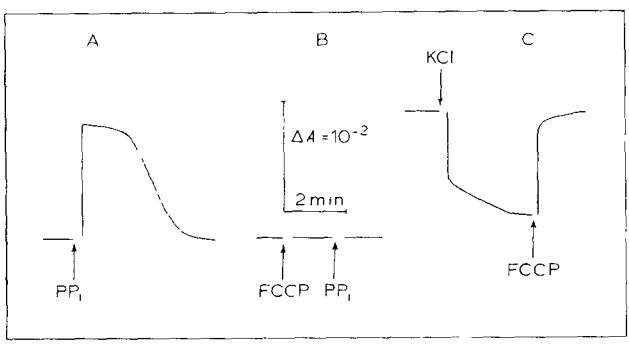



Fig 3. The kinetics of absorption changes induced by $PP_i$ or KCl and valinomycin in *R rubrum* chromatophores. A, an effect of $PP_i$ (50 $\mu$M) at 895 nm, $A_{880\ nm} = 1\ 5$ B, the same as A *plus* 3 $\mu$M FCCP. C, an effect of KCl (20 mM) at 851 nm in the presence of 0.5 $\mu$M valinomycin The concentration of added FCCP in C was 0 5 $\mu$M; $A_{880\ nm} = 6\ 4$

TABLE I

THE ABSORPTION CHANGES INDUCED BY DIFFERENT ENERGY SUBSTRATES IN *R RUBRUM* CHROMATOPHORES AT 895 nm IN THE DARK ($A_{880\ nm} = 3.3$)

| *Energy substrate* | *Concentration (mM)* | $1A_{895\ nm}\ 10^3$ |
|---|---|---|
| $PP_i$ | 0.1 | 7.7 |
| ATP | 2.0 | 5.8 |
| NADH | 2.0 | 3.5 |

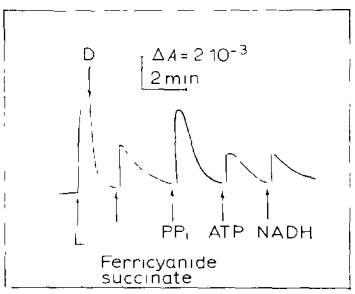



Fig. 4. The kinetics of energy-dependent changes of carotenoid absorption in *R rubrum* chromatophores at 570 nm. L, light, D, dark. Additions 7 $10^{-4}$ M succinate, 3 $10^{-4}$ M ferricyanide, $1.7 \cdot 10^{-5}$ M $PP_i$, 7 $10^{-5}$ M ATP and $3.3 \cdot 10^{-5}$ M NADH. $A_{570\ nm} = 0.65$.

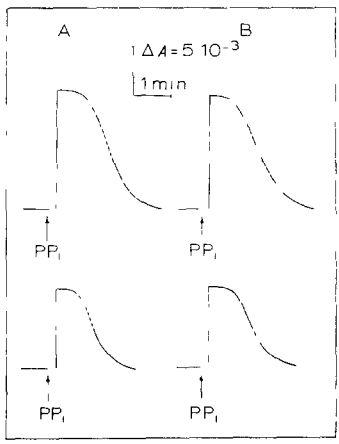
oxidation of NADH (Table I). But ATP and NADH are less effective in comparison with $PP_i$.

These results correlate with the effect of these compounds on the carotenoid band shifts of *R rubrum* chromatophores at 570 nm. Fig. 4 shows the carotenoid absorption changes induced by the light, oxidation of succinate by ferricyanide, hydrolysis of $PP_i$, ATP and oxidation of NADH by $O_2$ [9].

Fig. 3C illustrates that addition of KCl in the dark in the presence of valinomycin sharply reduces the absorption of bacteriochlorophyll in the region of the negative differential peak *i.e.* at 851 nm. This observation corresponds to the data of Jackson and Crofts[6] and Sherman and Clayton[7] on the carotenoid band shifts in the *Rps. spheroides* chromatophores. The addition of KCl in the presence of valinomycin generates the gradient of $K^+$ across chromatophores membranes. The inside of the membranes is positively charged. The uncoupler FCCP removes the effect of $K^+$ in the presence of valinomycin.

Thus, light in the presence of reduced TMPD, hydrolysis of $PP_i$ and ATP, electron transfer *via* energy coupling sites of the redox chain, as well as the combination of KCl and valinomycin cause a similar effect: the shifts of bacteriochlorophyll absorption bands. This means that the effect observed is due to the electrochemical potential generated across the membranes of chromatophores by a gradient of $H^+$ and $K^+$.

It is important that the absorption-band shifts of bacteriochlorophyll and carotenoids in the chromatophores are due to the electric constituent ($\Delta\Psi$) of the electrochemical gradient of $H^+$ and $K^+$ but not to the osmotic (concentration) constituent plotted as the transmembrane difference of the free ions, $H^+$ and $K^+$



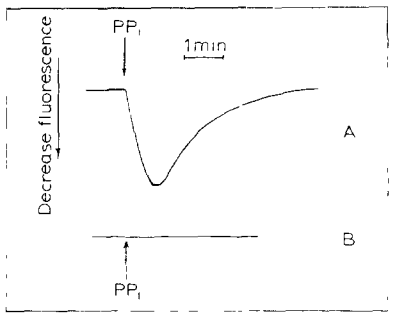




Fig. 5. Effect of KCl and nigericin on $PP_i$-induced changes of bacteriochlorophyll (upper curves) and carotenoid (bottom curves) absorption in *R. rubrum* chromatophores. A, absorption changes of bacteriochlorophyll at 895 nm and carotenoids at 572 nm in the presence of KCl (20 mM); B, the same as A *plus* nigericin (1.5 $\mu$g/ml). The concentration of $PP_i$ was 30 $\mu$M; $A_{880\ nm} = 5.0$.

Fig. 6. Effect of KCl and nigericin on $PP_i$-induced changes of atebrin fluorescence in *R. rubrum* chromatophores. A, in the presence KCl (20 mM); B, the same as A *plus* nigericin (1.5 $\mu$g/ml). Concentration of $PP_i$ was 30 $\mu$M; $A_{880\ nm} = 5.0$.

($\Delta$pH and $\Delta pK^+$) concentrations. Confirmation of this view may be found in the observation that the nigericin in the combination with KCl realizes the transmembrane exchange of $H^+$ on $K^+$, but does not influence the $PP_i$-induced shifts of bacteriochlorophyll and carotenoid absorption bands (Fig. 5). In contrast, fluorescence changes of atebrin, the indicator of $\Delta$pH on the membranes[14,15], are abolished by the combination of KCl and nigericin in the chromatophores energized by $PP_i$ (Fig. 6).

The light-induced shifts of bacteriochlorophyll absorption bands in the chromatophores are also observed in the absence of reduced TMPD. Under these conditions the negative peaks at 865 and 810 nm and positive peak at 790 nm are found in the difference spectrum (Fig. 7, Curve 1). An uncoupler FCCP reduces the amplitude of light-induced changes in the region of 865 nm and causes the appearance of a negative peak in the region of 895 nm (Fig. 7, Curve 2). This observation is consistent with the data of Okayama *et al.*[17]. The difference spectrum plotted by subtraction of Spectrum 1 from Spectrum 2 is similar to that induced by $PP_i$ or light in the presence of reduced TMPD (Fig. 7, Curve 3).

The fact that the blue shift of bacteriochlorophyll and the negative peak at 865 nm (Fig. 7, Curve 2) are retained in the presence of FCCP may indicate that the effects observed are not only due to the transmembrane electric potential difference. It may well be that the additional contribution gives the light-induced generation of a local electric field arising as a result of the separation of charges between the bacteriochlorophyll reaction center (P) and the primary electron acceptor (A). This assumption is in agreement with the data of the carotenoid shift in the chromatophores of *R. rubrum*[9].

In such a case the photo-bleaching observed at 865 nm in the absence of the

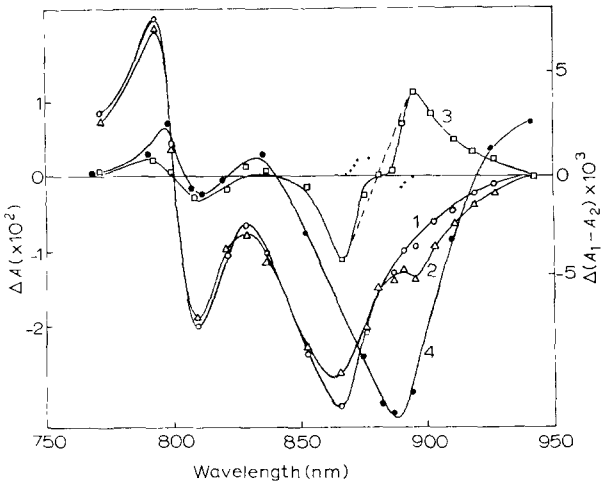



Fig. 7 The spectra of absorption changes induced by light or ferricyanide in *R. rubrum* chromatophores 1, light-induced changes; $A_{880\ nm} = 2\ 24$. 2, the same as 1 *plus* 20 $\mu$M FCCP 3, Spectrum 1 *minus* Spectrum 2. 4, changes induced by ferricyanide (0.24 mM) in the dark. $A_{880\ nm} = 0.94$

reduced TMPD may be due to the superposition of (a) the red shift of the bacteriochlorophyll absorption band caused by the transmembrane potential and the local electric field, (b) the photooxidation of the bacteriochlorophyll reaction centers absorbing in the region of 890 nm.

It is known that the light-induced changes of absorption connected with the transition of reaction center in the oxidized state are observed only at the definite values of medium redox potential[18]. In a strong reducing or oxidizing environment the reaction centers are not accessible to oxidation because the components of the electron transfer chain are in a reduced state (in the first place) or the reaction centers are chemically oxidized and are not able to respond to light (in the second place).

In our experiments $TMPDH_2$ effectively reduces the reaction centers and produces a drop in the absorption changes upon continuous illumination (Fig. 1, Curve 2). Evidently, such an effect reduces the tension of the local electric field arising upon the photooxidation of the reaction center. That is why only the effect of transmembrane potential linked with the mechanism of energy coupling is observed (Fig. 1,

TABLE II

THE EFFECT OF FERRICYANIDE (0.5 mM) AND DITHIONITE (1 mg/ml) ON THE ABSORPTION CHANGES ($\Delta A \times 10^3$) INDUCED BY LIGHT OR $PP_i$ (20 $\mu$M) IN *R. RUBRUM* CHROMATOPHORES AT 865 AND 810 nm ($A_{880\ nm} = 2\ 6$)

| | *Light* | | *$PP_i$* | |
|---|---|---|---|---|
| | *865 nm* | *810 nm* | *865 nm* | *810 nm* |
| Control | 80 | 50 | 21 | 2 9 |
| Ferricyanide | 42 | 24 | 28 | 3 9 |
| Dithionite | 3 6 | 4 7 | 21 | 2 9 |

Curve 1). This conclusion is also in agreement with the data of Table II. The addition of ferricyanide or dithionite to the chromatophores suspension has no influence on the $PP_1$-induced dark shifts of bacteriochlorophyll absorption bands in the region of 810 and 865 nm, but it depresses the light-induced responses. These results also indicate that the transmembrane potential generation by $PP_1$ is independent of the electron transfer *via* energy-coupling sites of chromatophores redox chain.

The steady-state level of the oxidized reaction centers increases upon continuous illumination in the absence of reducing or oxidizing agents, when the components of the electron transfer chain are in a partly oxidized state. This leads to the increase of the local electric field effect on the spectral shifts of bacteriochlorophyll. The superposition of (1) the red bacteriochlorophyll shift due to transmembrane potential and the local electric field and (2) photo-bleaching of the reaction centers in the region of 890 nm makes the appearance of the overall absorption change with the negative peak at 865 nm possible.

The assumption that the true bleaching of the reaction centers is localized not at 865 nm, but in a more long-wave region, is consistent with the data in Fig. 7, Curve 4. In the dark spectrum of the absorption changes induced by ferricyanide the blue shift of bacteriochlorophyll is found and the negative peak at 890 nm (but not at 865 nm as is observed in the case of light-induced absorption changes).

There is supporting evidence for the fact that the absorption band of the reaction centers is situated in the 890-nm region. The energy-linked changes in bacteriochlorophyll absorption have a complicated nature in the region of 850–900 nm (Fig. 7, Curve 3). These absorption changes represent a superposition of two effects: a red shift of light-harvesting bacteriochlorophyll (dashed line) and blue shift (dotted line). According to data (Clayton, R. K., personal communication) the blue shift observed is due to the absorption band shift of the reaction centers (P) which are at $PA^-$ state, where $A^-$ is the reduced primary electron acceptor. Such an effect is observed only for some chromatophore preparations. From our calculation the blue shift of the reaction centers absorption band reaches the value of 10–15 nm against the isosbestic point. Thus the absorption peak of the reaction centers is localized at 890 nm.

The ratio of the effects of transmembrane and local electric fields on the spectral characteristics of bacteriochlorophyll in the chromatophores depends on the intensity of the actinic light. A "symmetrical" red shift of the bacteriochlorophyll with peaks at 895 and at 865 nm is found upon relatively low intensity of light in the presence of $TMPDH_2$ (Fig. 8, Curves 1 and 2).

The level of the absorption changes decreases at 895 nm and increases at 865 nm at high intensities: the spectrum of the light-induced changes becomes similar to that in the absence of $TMPDH_2$. The light curves of the absorption changes at 810 nm, connected with the blue shift of bacteriochlorophyll, are very similar to those at 865 nm (Fig. 8, Curve 3).

The observed light-curve differences may be explained by the increase in the steady-state level photooxidized reaction centers upon the increase of actinic light intensity. The effect of a local electric field may be predominant under these conditions.

Summarizing the results obtained we may conclude that the complicated nature of bacteriochlorophyll light-induced changes is linked with the superposition of two

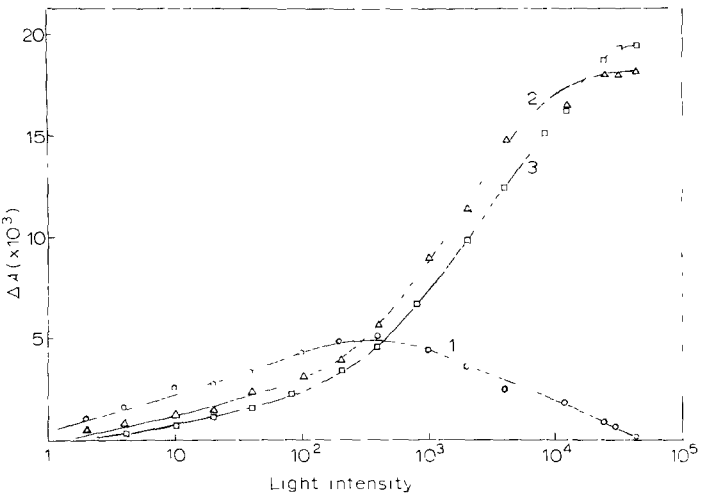



Fig 8 The effect of actinic light intensity (relative units, $\lambda > 700$ nm) on the absorption changes in *R. rubrum* chromatophores in the presence of TMPD (0.1 mM) and ascorbate (1 mM). 1, at 895 nm, 2, at 865 nm, 3, at 810 nm $A_{880\ nm} = 1.5$

effects: (1) the change in the redox level of the bacteriochlorophyll reaction center and (2) the change in the bacteriochlorophyll absorption spectrum upon the appearance of the electric field in the membranes of chromatophores (the electrochromic effect).

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