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LIGHT- AND CHEMICALLY-INDUCED OXIDATION–REDUCTION REACTIONS IN CHROMATOPHORE FRACTIONS OF *RHODOSPIRILLUM RUBRUM*

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SUMMARY

Chromatophore fractions of *Rhodospirillum rubrum* were prepared by centrifugation of a 'classical' chromatophore preparation in a sucrose density gradient. Most of the experiments were carried out with a fraction separating out at about 23 % sucrose. Although no light-induced reactions of cytochromes could be detected, the presence of two bound cytochromes, C422 and C428, in this fraction was indicated by oxidation–reduction reactions induced by KIO_4 and dithionite. Cytochrome C422 appeared to be the 'high-potential' cytochrome detected earlier in preparations from this organism. Titration of chemically-induced absorbance changes due to a reduction of oxidized cytochrome C428 has led to an estimated redox midpoint potential of $E_{m7.5} = -0.01$ V for this component in a four-electron transport reaction.

Light-induced absorbance changes in the near-infrared spectral region measured in the light chromatophore fraction at different environmental redox potentials suggested a bacteriochlorophyll component, P', with spectral characteristics which were different from those of the high-potential bacteriochlorophyll component P890. The spectral shifts observed upon oxidation, either by light or by air were a bleaching in the 860–880-nm spectral region and an appearance of an absorption band at about 900 nm. The component could be oxidized and reduced reversibly and had an estimated midpoint potential of +0.06 V in a one-electron reaction.

INTRODUCTION

It seems to be generally accepted (*cf.* ref. 1) that photosynthetic electron transport in most, if not all, photosynthetic bacteria occurs in two distinct pathways, a non-cyclic one which is coupled to substrate dehydrogenation and a cyclic one which is coupled to ATP formation. In a number of recent papers[2–7] experiments have been reported which suggested that in the photosynthetic bacteria *Chromatium* and *Rhodospirillum rubrum* the two electron transport chains are driven by two distinct light reactions. Different action spectra for the light-induced oxidation of cytochromes have been reported[2,5] for both species. Moreover, in whole cells of *R. rubrum* two types of light-induced absorbance changes in the near-infrared spectral region could be detected[6,7]. One of these could be related to the cyclic electron trans-

port and showed a decrease in absorbance centered at about 880 nm accompanied by a blue shift of an absorption band at about 805 nm. This type of light-induced absorbance change has been observed before[8–10] and attributed to the light-induced oxidation of a specialized bacteriochlorophyll component (P890) in so-called reaction centers. The other type of light-induced absorbance change which appeared to be connected with the non-cyclic electron flow, showed only an absorbance decrease around 880 nm with little or no shift of the 805–nm band. The appearance of an absorption band at about 900 nm often was observed together with this kind of absorbance change (C. SYBESMA, unpublished observation). A similar band was observed in various other purple photosynthetic bacteria[3,11,12].

CUSANOVICH *et al.*[3] recently reported a study of light–induced absorbance changes as related to the redox potential of the suspension medium in a chromatophore preparation of *Chromatium* strain D. Midpoint potentials at a pH of 7.5 were estimated for the C555 cytochrome in the cyclic electron transport chain (+0.34 V), the reaction center component P890 (+0.49 V) and a two-electron acceptor for the substrate-coupled C552 cytochrome (–0.14 V). Photophosphorylation appeared to occur predominantly in the cyclic system in which both P890 and C555 cytochrome are involved[4]. Light-induced absorbance changes measured in a high-potential environment were attributed to this system. The dramatically different light-induced absorbance changes occurring in a low-potential environment were attributed to a non-cyclic electron flow, mediated by different components, from external substrates to pyridine nucleotide.

In *R. rubrum* chromatophores, a midpoint potential of 0.44 V for the reaction center component P890 has been estimated by KUNTZ *et al.*[13]. HORIO AND YAMASHITA[14] reported a midpoint potential of +0.31 V for the cyclic cytochrome C422 in chromatophores of this organism. So far no data has been reported for the non-cyclic cytochrome C428 in this organism. The present paper reports a study of some redox properties of the reaction center components and cytochromes in chromatophore preparations from this organism.

MATERIALS AND METHODS

R. rubrum strain IV (Giesbrecht) was grown as described previously[5,7] in a medium containing malate and glutamate. Aliquots were taken from 3-day-old cultures and chromatophore preparations were prepared by a technique substantially similar to the one used by CUSANOVICH and KAMEN[15]. RbCl-treated classical chromatophores were centrifuged in a sucrose density gradient. A heavy fraction separated out between 37.5 and 44.0 % sucrose and a light fraction between 19.0 and 28.0 % sucrose. The absorption spectra of the classical chromatophores and the two sucrose density gradient particles were not very different from each other. Light-induced spectral changes were measured with all three preparations; the measurements of chemically-induced oxidation–reduction reactions and the redox titrations were carried out with the light fraction only. All types of particles were suspended in a phosphate–sucrose buffer at a pH of 7.5 (0.1 M phosphate buffer and 10 % sucrose).

Light-induced changes in absorbance were measured with a split-beam difference spectrophotometer described earlier[5,6]. Difference spectra of chemically-induced spectral changes were measured in a Cary 14R recording spectrophotometer. Cuvettes

of 10-mm optical pathlength with ground glass stoppers were used. Aerobiosis was induced by bubbling air into the cuvette with a hypodermic syringe. The measurements of difference spectra in the Cary 14R spectrophotometer were carried out with a 0.0–0.1, 0.1–0.2 absorbance slidewire. Each experiment included a control run of equal suspensions in both the reference and the sample compartment in order to establish a base line.

Redox potentials were measured with a Century model Beckman pH meter with a combination platinum electrode. Colored redox indicators were used for the titrations, methylene blue for the lower potentials and brilliant cresyl blue for the higher potentials. The redox indicators were standardized with the combination platinum electrode which was calibrated against a saturated quinhydrone solution. The potentials of the chromatophore suspensions were adjusted in the presence of one of the redox indicators by addition of $Na_2S_2O_4$ or $K_3Fe(CN)_6$ or the system was allowed to equilibrate.

RESULTS

All three fractions, suspended in the phosphate–sucrose buffer without any additions, exhibited light-induced spectral changes which were not substantially different in aerobic suspensions as compared with anaerobic suspensions. In the near-infrared spectral region these changes consist of a decrease of absorbance centered at about 865 nm and a blue shift of a band around 800 nm. In the Soret spectral region a light-induced increase of absorbance centered at about 435 nm could be observed in all three fractions. As shown in Fig. 1, the spectral characteristics of this change were slightly different in the light fraction as compared with either the heavy fraction or the classical chromatophore. When the light-*minus*-dark spectrum of the light fraction was subtracted from the light-*minus*-dark spectrum of either a classical chromatophore preparation or the heavy fraction, a typical oxidation-*minus*-reduction spectrum of a cytochrome appeared. The spectral position of the Soret-trough suggested this cytochrome to be cytochrome C422, the cytochrome in the cyclic electron transport chain. Since this cytochrome most likely is oxidized rather than reduced by light, this result may indicate that more cytochrome C422 per reaction center is bound to the light particles than to the heavy particles or the classical chromatophores. No indication was seen for a reaction of the other photosynthetic cytochrome-like component, C428. A light-induced spectral shift due to the oxidation of cytochrome C428 possibly could be masked by the large light-induced increase of absorbance at 435 nm. However, an alternative explanation may be that cytochrome C428 is already in the oxidized state. The measured redox potential of an anaerobic suspension of light-particles in phosphate–sucrose buffer was +0.23 V. It is also known that in whole cells of *R. rubrum* the light-induced oxidation of cytochrome C428 ceases under aerobic conditions.

A number of experiments was carried out in which suspensions of the light chromatophore fraction in different chemical environments were compared with each other in the Cary 14R spectrophotometer. Fig. 2 shows a typical example of a series of such experiments. When an aerobic suspension of the light fraction was compared with an anaerobic suspension of that fraction, the difference spectrum showed no features in the Soret region. However, in the near-infrared spectral region

the aerobic-*minus*-anaerobic spectrum showed a trough in the spectral range from 860–880 nm and a peak at wavelengths above 900 nm (solid lines). If the same anaerobic sample was reduced by dithionite and compared with the aerobic sample, a trough appeared at 428 and a peak at 414 nm suggesting involvement of cytochrome C428, whereas the spectrum in the near-infrared remained unchanged.

If the near-infrared spectral changes are interpreted as due to oxidation–reduction reactions of a redox intermediate which we will call P′, the following conclusions can be made from these results: cytochrome C428 is in the oxidized

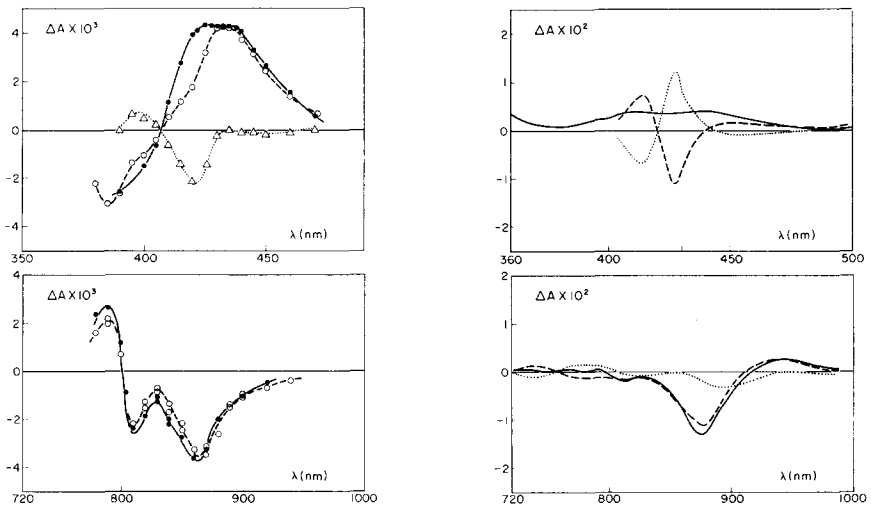

Fig. 1. Light-*minus*-dark difference spectra of absorbance changes in anaerobic suspensions of *R. rubrum* chromatophore fractions. ●———●, classical chromatophore fraction; ○-----○, light fraction (see text); △·····△, difference between light and the classical chromatophore fraction. The intensity of the actinic light was saturating. The absorbance at 880 nm of both suspensions was about 0.6.

Fig. 2. Difference spectra measured in suspensions of the light chromatophore fraction of *R. rubrum* (see text) in different chemical environments. ———, aerobic-*minus*-anaerobic difference spectrum; - - - -, aerobic-*minus*-dithionite-reduced difference spectrum; ·····, dithionite-reduced-*minus*-anaerobic difference spectrum. The absorbance at 880 nm of the suspensions was about 2.

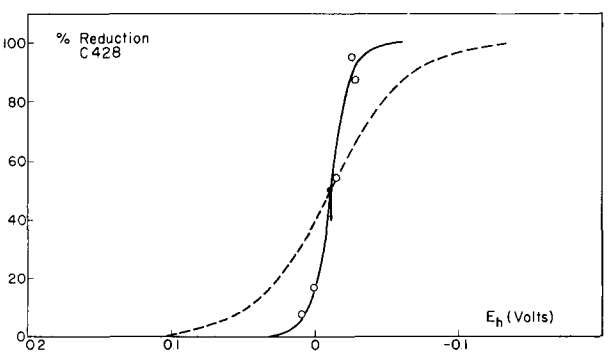

Fig. 3. Titration of the absorbance change at 428 nm induced by addition of dithionite in a suspension of the light chromatophore fraction of *R. rubrum* (see text), using methylene blue as redox indicator.

state, in both aerobic and anaerobic suspensions. Dithionite reduces this component. P′ is in the reduced state in anaerobic suspensions. O_2 is able to oxidize it when aerobic conditions are established. Dithionite keeps it reduced.

The absorption change, presumably due to the reduction of cytochrome C428 by dithionite was titrated using methylene blue as the redox indicator. Fig. 3 shows the results from which a midpoint potential of −0.01 V for this component can be concluded. The experimental points seemed to fit best the curve for a four-electron transport reaction.

The absorption changes in the near-infrared spectral region were titrated using brilliant cresyl blue as the indicator. Titration of the 900-nm band did not give results different from the titration of the 870-nm band, indicating that these changes are due to an oxidation–reduction reaction of one component. The results are shown in Fig. 4. The midpoint potential is +0.06 V and the data fit a one-electron transport reaction fairly well.

No indications were found for oxidation–reduction reactions of cytochrome C422 and the reaction center component associated with the light-induced blue shift of the 800-nm band in this potential range. The addition of a strong oxidant like KIO_4 resulted in the oxidation of these components, as shown in Fig. 5. In the Soret spectral region the trough at 422 nm and the peak at 410 nm suggested the oxidation of cytochrome C422. In addition a relatively broad peak at about 440 nm appeared. The near-infrared spectral region showed a large bleaching at about 885 nm and a blue shift of a band at about 800 nm. The near-infrared difference spectra differ from the light-induced difference spectra (*cf.* Fig. 1) in that the chemically-induced spectra are much larger than the light-induced spectra, the ratio of the amount of bleaching and the blue shift of the 800-nm band is larger in the chemically-induced reaction than in the light-induced reaction and, that the chemically-induced bleaching is centered at 885 nm while the light-induced bleaching is centered at 865 nm. These differences could be due to a partial oxidation of the bulk bacteriochlorophyll by the chemical oxidant. If ferricyanide was used instead of the periodate in such a concentration as to poise the suspension at +0.45 V, the ratio of the bleaching and the 800-nm blue shift indeed came closer to the value measured in the light-

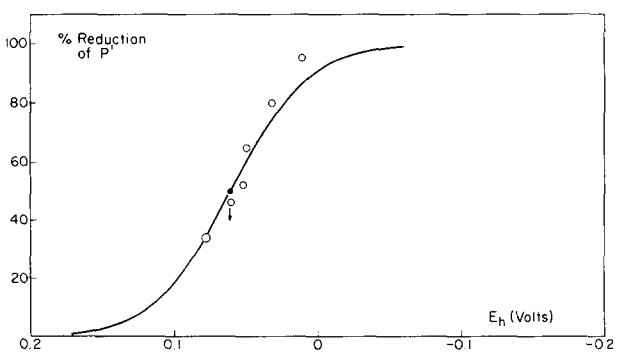

Fig. 4. Titration of the chemically (dithionite)-induced changes of the near-infrared absorbance of a suspension of the light chromatophore fraction of *R. rubrum* using brilliant cresyl blue as redox indicator. Points are the sum of the absolute values of the bleaching at 865 nm and the increase of absorbance at 900 nm. ———, theoretical curve for a one-electron transport reaction.

induced reaction. Moreover, the result of a subtraction of the chemically-induced difference spectrum from the light-induced difference spectrum is a spectrum similar to the absorption spectrum of the suspension.

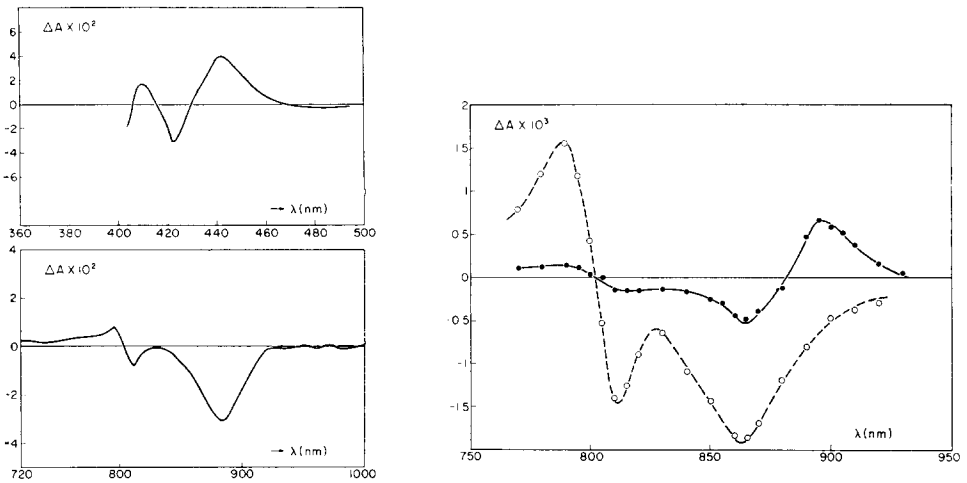

Fig. 5. Difference spectra of absorbance changes induced by KIO_4 in a suspension of the light chromatophore fraction of *R. rubrum* (see text). The absorbance at 880 nm of the suspensions was about 2.

Fig. 6. Light-*minus*-dark difference spectra of absorbance changes in suspensions set at different redox potentials of the light chromatophore fraction of *R. rubrum* (see text). ●———●, difference spectrum measured in a suspension set at −0.01 V; O- - - -O, difference spectrum measured in a suspension set at +0.2 V. The absorbance at 880 nm of the suspensions was about 0.6.

Fig. 6 shows light-*minus*-dark difference spectra measured in a sample of the light fraction suspended in a phosphate–sucrose buffer medium poised at two different potentials. The solid curve is the spectrum measured at −0.01 V. It shows a light-induced bleaching centered at 863 nm, and the appearance of a band at 895 nm but hardly any feature around 800 nm. The dashed line is the spectrum measured when the suspension was allowed to equilibrate at a potential of about 0.2 V. In the 800-nm region a substantial blue shift was induced in addition to a bleaching at 865 nm. No increase of absorbance was detected at wavelengths higher than 880 nm. In a suspension in which the potential was set at +0.03 V the light-*minus*-dark difference spectrum (not shown) included all three features. In the Soret spectral region, a light-induced increase of absorbance with a complicated spectral appearance was seen at all environmental potentials.

DISCUSSION

Light-induced reactions of cytochromes could not be detected directly in any of the three chromatophore fractions. Yet, the chemically-induced absorbance changes measured in the light chromatophore fraction indicated clearly the presence of the bound cytochromes C422 and C428 in this fraction. Cytochrome C428 appeared to be present in the oxidized state, independent of the presence or absence of O_2 when the redox potential of the environment was set at about 0.23 V. The measured

midpoint potential of –0.01 V for this cytochrome is in agreement with this situation. Titration of the chemically-induced absorbance change at 428 nm agrees with a four-electron transport reaction. Multi-electron transport reactions for non-cyclic cytochromes have been detected in other purple bacteria as well (*cf.* ref. 1).

The addition of KIO_4, setting the environmental potential at a high level, resulted in spectral phenomena typical for the oxidation of a cytochrome C422 and, in addition, the appearance of an absorption band at about 440 nm. The exact spectral position of this band is uncertain because of interference with the absorbance changes due to the oxidation of cytochrome C422. It is possible that this absorption band is due to the oxidation of a component which also reacts with light. The spectral phenomena seen upon illumination of the chromatophore suspension, however, are much more complex, and possibly are masking the light-induced oxidation of cytochrome C422. In fact, the only indication of a light-induced reaction of this cytochrome is the appearance of a typical cytochrome oxidation-*minus*-reduction spectrum when the light-*minus*-dark spectrum of a light chromatophore fraction is compared with a light-*minus*-dark spectrum of a classical or heavy chromatophore preparation (*cf.* Fig. 1). The fact that the light-induced spectral phenomena in the light chromatophore fraction in this spectral region were persistent in different environmental redox potentials could indicate that at least part of these phenomena are not due to light-induced oxidation–reduction reactions at all.

Evidence for a non-cyclic light-induced electron transport involving the cytochrome C428 and mediated by a reaction center different from the one which drives the cyclic electron transport chain has been reported before[5–7]. The present results are not in disagreement with these results, although a close relationship between the two components could not be detected in the light chromatophore fraction. The data indicates that the redox state of the component P′ exhibiting the spectral change at low potential can be changed by altering the degree of anaerobiosis whereas the redox state of the cytochrome C428 remains unchanged. This agrees with the fact that cytochrome C428 has a lower redox potential than the near-infrared component. However, the redox potentials of both components are in the same (relatively low) range and the light-induced near-infrared absorbance changes in a low-potential environment showed distinct spectral differences as compared with those measured at a higher environmental potential. Thus, in view of the previously reported evidence, it seems reasonable to assume that these changes are due to the oxidation–reduction reactions of a component P′ (possibly bacteriochlorophyll) of a reaction center which operates at a redox level lower than that of the well known reaction center component P890. The spectral characteristics of the oxidation of this component are a bleaching in the 860–880-nm spectral region and the appearance of an absorption band around 900 nm. The titration of these absorbance changes indicated that both are due to a redox reaction of one component. The absorbance changes may or may not indicate a red shift of a bacteriochlorophyll absorption band.

Although proper reservation should be kept in mind when an extrapolation is made from the phenomena observed in the chromatophore preparations to the situation in living whole cells, the presented results support a model[17] in which photosynthetic electron transport in *R. rubrum* is driven by two distinct light reactions, one operating at a relatively high potential and cyclic in nature and the other operating at a lower potential and coupled to substrate dehydrogenation. The potential

range bridged by the latter reaction is sufficient to account for a direct light-induced electron transport from substrates like malate or succinate to nicotinamide-adenine dinucleotide[16].

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