Bioenergetics (1973) 4, 391-395

The Bacteriochlorophyll Absorption Band Shifts Linked with the Energy State of Photosynthetic Bacteria Membranes

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Received 2 October 1972

Abstract

The uncoupler of photophosphorylation FCCP inhibits the light-induced changes in absorbancy for *Rhodospirillum rubrum*, *Ectothiorhodospira shaposhnikovii* and *Chromatium minutissimum* cells in anaerobic conditions. These changes are associated with the shifts of bacteriochlorophyll absorption bands. The superposition of these spectral shifts and the photobleaching of reaction centers P890 is observed in aerobic conditions.

The light-induced shifts of bacteriochlorophyll absorption bands are suggested to be due to the electrochemical transmembrane potential and local electric field arising as a result of the primary separation of opposite charges.

Introduction

The shifts of bacteriochlorophyll and carotenoid absorption bands together with the photooxidation of reaction centers P890 (P870) are observed in a number of purple bacteria [1, 2,]. Several different suggestions have been made about the nature of these shifts. Some authors [2] associate the spectral shifts with changes in a conformation

Abbreviations: FCCP, carbonylcyanide-p-trifluoromethoxy phenyl-hydrazone; TMPD, tetramethyl-p-phenylenediamine.

of the protein-pigment structure. Others [3] believe the shifts of bacteriochlorophyll absorption bands are connected with the functioning of the reaction centers P905 in non-cyclic electron transfer chains.

It has been demonstrated that the carotenoid shifts may be caused by the electrochemical potential of H^+ or K^+ ions generated across the membranes of *Rhodopseudomonas spheroides* chromatophores [4, 5]. Similar results were obtained with chloroplasts [6] and *R. rubrum* chromatophores [7].

The purpose of the present communication was to set up a comparative study of the shifts of bacteriochlorophyll absorption bands in the cells of non-sulphur and sulphur purple bacteria.

Methods

Three- to five-day-old cultures of purple sulphur bacteria *Ectothiorhodospira shaposhnikovii* and *Chromatium minutissimum* as well as non-sulphur bacteria *Rhodospirillum rubrum* were used in the experiments. The sulphur bacteria were grown under anaerobic conditions on modified Larsen's medium [8], and *R. rubrum*—in the medium of Bose *et al.* [9] Both aerobic and anaerobic cell suspensions were used. The bacteria were aerated by blowing air. Anaerobic conditions were obtained by incubation of cell suspensions under vaseline oil.

The light-induced bacteriochlorophyll absorption changes were measured with the single-beam spectrophotometer [10] in the near infrared region. Saturating intensity of actinic light with λ more than 700 nm was obtained from a tungsten lamp.

The concentration of FCCP was 10^{-5} M in all experiments.

Results

The absorption changes are observed under illumination of the anaerobic *R. rubrum* cell suspensions (Fig. 1A, curve 2). These changes are caused, apparently, by absorption shifts of the P800 reaction center component and B880 bacteriochlorophyll spectral form to the short and long wave region of spectrum respectively (Fig. 1A, curve 1). Similar changes are observed in *R. rubrum* chromatophores. This effect may be induced by light when the incubation medium contains TMPD and ascorbate as well as in the dark by addition of inorganic pyrophosphate, ATP, NADH or K^+ ions in the presence of valinomycin [7]. The uncoupler of photophosphorylation FCCP removes the absorption changes observed with bacterial cells in anaerobic conditions (Fig. 1A, curve 3).

The "aerobic" spectra of light-induced absorption changes differ

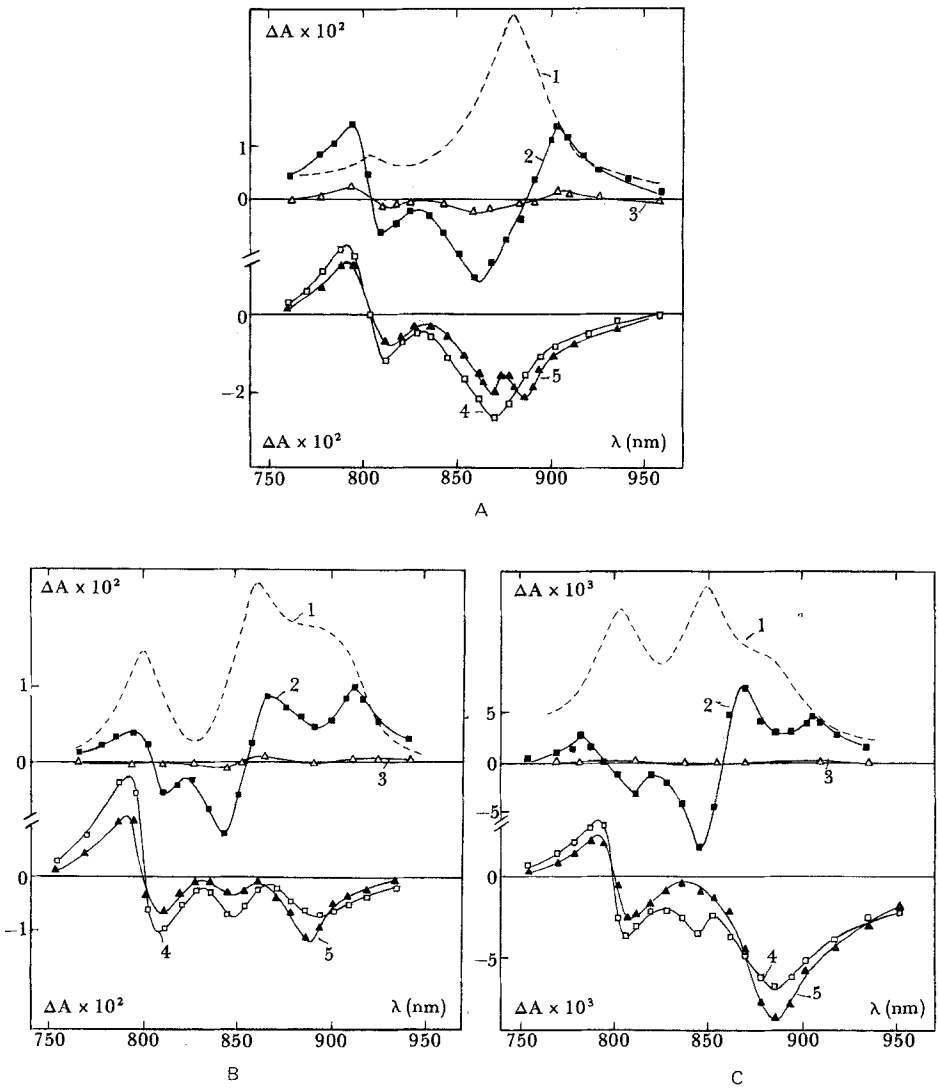

Figure 1. Absolute absorption and its light-induced changes spectra for whole cells of purple bacteria. I—absorption spectrum. 2,4—light-minus-dark differential spectra in anaerobic and aerobic conditions respectively. 3,5—the same as 2,4, but in the presence of FCCP. A— for *R. rubrum*. B— for *E. shaposhnikovii*. C— for *Chr. minutissimum*.

significantly from "anaerobic" ones (Fig. 1A, curve 4). In this case the photobleaching takes place at 870 nm which is accompanied by a spectral shift of P800. In the presence of FCCP the absorption decrease at 870 nm and shift of P800 are reduced and new negative maximum at 885-890 nm appears (Fig. 1A, curve 5). Both the absolute absorption spectrum and its light-induced changes are similar for *E. shaposhnikovii* and *Chr. minutissimum* cells (Fig. 1B, C; curves 1, 2). The changes connected with the shifts of P800, B850 and apparently B890 are suppressed by FCCP (curve 3).

In aerobic conditions light-minus-dark differential spectra for both bacteria are characterized by photobleachings centered at 890 and 850 nm as well as by the blue shift of P800 (curve 4). In the presence of FCCP the spectral shifts of P800 and photobleaching at 850 nm decrease while photobleaching at 890 nm increases (curve 5).

Discussion

The data obtained demonstrate that in anaerobic cell suspensions of the nonsulphur purple bacteria *R. rubrum* as well as in aerobic suspensions of *R. rubrum* chromatophores in the presence of reduced TMPD [7] the light-induced shifts of bacteriochlorophyll absorption bands sensitive to FCCP are found. We have also observed such absorption changes in anaerobic cell suspensions of sulphur purple bacteria *Chr. minutissimum* and *E. shaposhnikovii*. Recently it has been shown that similar changes in the *R. rubrum* chromatophores can be induced in the dark by hydrolysis of inorganic pyrophosphate and ATP, by oxidation of NADH and by addition of KCl in the presence of valinomycin.

The analysis of the data obtained leads to the conclusion that the shifts of bacteriochlorophyll absorption bands observed with purple bacteria in anaerobic conditions are induced by the transmembrane electrochemical potential of H^+ ions.

It appears reasonable to assume that a local electric field insensitive to FCCP arises near the reaction centers in addition to the transmembrane potential of H^+ ions. The local electric field may be caused by the primary light-induced separation of opposite charges between the bacteriochlorophyll reaction center (P) and the primary electron acceptor and may exist long enough in suspensions of aerobic cells or chromatophores in the case when a high oxidation level of the reaction center is observed. The decrease of local electric field tension may be caused in anaerobic cells by rapid P^+ reduction by intracellular oxidizable substrates or in aerobic chromatophores in the presence of reduced TMPD.

Thus, the bacteriochlorophyll absorption shifts observed in anaerobic conditions are due to the electric potential difference applied to the

chromatophore membranes. The aerobic absorption changes of bacteriochlorophyll appears to be caused by three phenomena: 1—the photooxidation of reaction centers, 2—the local electric field of the charges separated in the primary light-induced act, 3—the transmembrane electrochemical potential.

Acknowledgement

We thank Dr. A. Yu. Borisov from Bioenergetics Department of Moscow State University for many helpful discussions.

References

1. J. Amesz and W. J. Vredenberg, in: *Current in Photosynthesis,* Dounker Publ. Rotterdam (1966) 75.
2. W. J. Vredenberg, J. Amesz and L. N. M. Duysens, *Biochem. Biophys. Res. Commun.*, 18 (1965) 435.
3. M. A. Cusanovich, R. G. Bartsch and M. D. Kamen, *Biochim. Biophys. Acta,* 153 (1968) 397.
4. J. B. Jackson and A. R. Crofts, *FEBS Letters,* 4 (1969) 185.
5. L. A. Sherman and R. K. Clayton, *FEBS Letters,* 22 (1972) 127.
6. H. M. Emrich, W. Junge and H. T. Witt, *Z. Naturforsch.*, 146 (1969) 1428.
7. E. L. Barsky and V. D. Samuilov, *Biochimia (USSR),* 37 (1972) 1005.
8. E. N. Kondrat'eva, in: *Photosynthesirujuschie bacterii,* Publ. Acad. Sci., USSR, Moscow (1963) 29.
9. J. G. Ormerod, K. S. Ormerod and H. Gest, *Arch. Biochim. Biophys.*, 14 (1961) 449.
10. E. L. Barsky and A. Yu. Borisov, *J. Bioenergetics,* 2 (1971) 275.