# ON THE NATURE OF THE LIGHT-INDUCED BACTERIOCHLOROPHYLL ABSORBANCE CHANGES IN CHROMATOPHORES OF *RHODOSPIRILLUM RUBRUM*

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## 1. Introduction

The nature of the light-induced red-shift of the major infrared absorption band of bacteriochlorophyll in photosynthesizing purple bacteria [1,2] is widely discussed. Some authors associate this spectral shift with chemical reactions of the pigment [3–5], while others relate it to physico-chemical changes in the pigment surroundings [2,6–8].

The bacteriochlorophyll red-shift is inhibited by uncouplers [4,7–9] and can be induced in the dark by the addition of ATP, $PP_i$ and KCl to *R. rubrum* chromatophores in the presence of valinomycin [9]. After light illumination or such energizing chemical treatment the electric potential difference is generated across chromatophore membrane [10], which suggests that the bacteriochlorophyll red-shift is the result of the electric field action on the pigment molecules (Stark or electrochromic effect).

The light-induced electric field includes the local component closely associated with the photochemical separation of electric charge in the reaction centres and the transmembrane component related to the proton gradient formation across the membrane. Our paper is concerned with the contribution the above mentioned electric field components make to the bacteriochlorophyll spectral shift. To clear up this problem absorption difference spectra (light *minus* dark) of *R. rubrum* chromatophores were investigated under different temperature conditions in the presence or absence of the electron donor (*N*, *N*, *N*′, *N*′-tetramethyl-*p*-phenylenediamine, TMPD-$H_2$). It is the purpose of the present communication to demonstrate that the bacteriochlorophyll red-shift observed at 297°K in the presence of TMPD-$H_2$ is due mainly to the transmembrane electric field. We will also show that at 297°K without TMPD-$H_2$ and at 80°K, whether TMPD-$H_2$ is present or not in the reaction mixture, difference spectra demonstrate absorbance changes which correspond both to the oxidation of the photoactive bacteriochlorophyll and the red-shift in the absorption band of bulk bacteriochlorophyll B 880 caused by local field of separated electric charge in the reaction centres.

## 2. Materials and methods

Methods for cultivation of *R. rubrum* bacteria cells and preparation of a chromatophore fraction have been described elsewhere [10]. Incubation mixture contained 0.25 M sucrose, 50 mM Tris-HCl buffer (pH 7.6) and 5 mM $MgCl_2$. *N*, *N*, *N*′, *N*′-tetramethyl-*p*-phenylenediamine reduced by 0.1 mM of ascorbate was used as the exogenous electron donor.

Light-induced absorbance changes were measured in a single-beam difference spectrophotometer with a 5 msec response time [11]. The apparatus was equipped with a low temperature attachment [12]. In all experiments at 297°K the intensity of the monochromatic measuring light beam did not exceed 5 erg $cm^{-2}$ $sec^{-1}$. The increase of the measuring light intensity due to the decrease of the transmittance of frozen samples leads both to the underestimation of

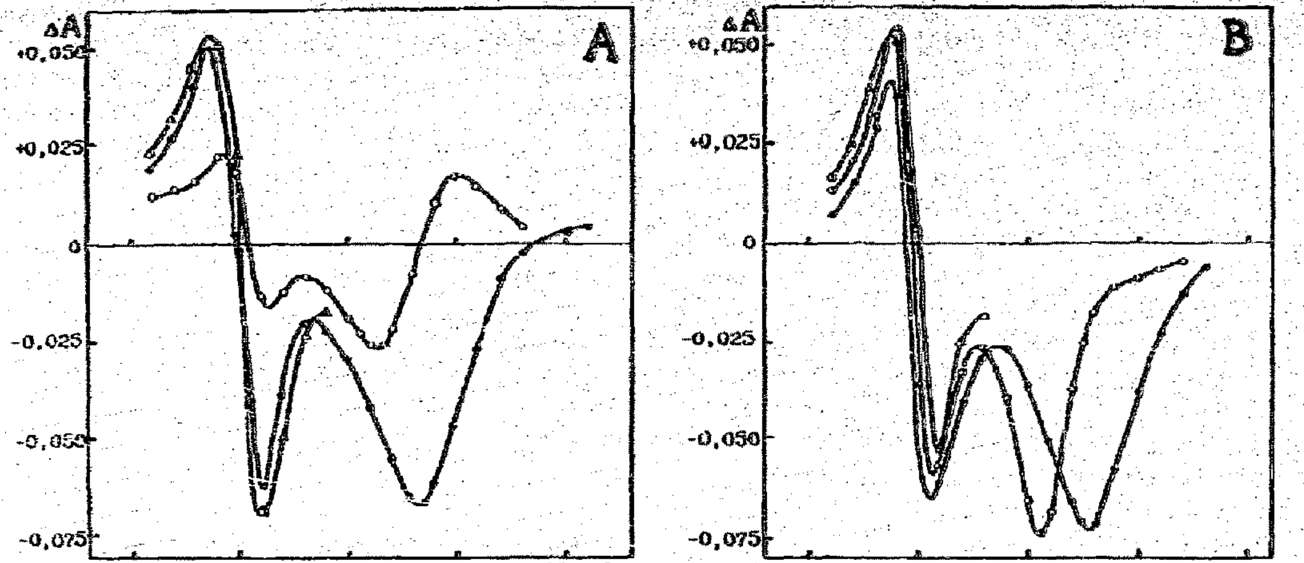

Fig. 1. Absorption difference spectra (light *minus* dark) of *R. rubrum* chromatophores. A) No addition; B) in the presence of TMPD-$H_2$. Open circles – at 297°K; closed circles – 80°K; triangles – at 80°K measured with the monochromator placed between the sample and the photomultiplier. The reaction mixture contained: 0.03 ml of stock chromatophore suspension in the incubation medium, 0.04 ml of 20 mM TMPD-$H_2$; total sample volume was adjusted to 2 ml with Tris-HCl buffer (pH 7.6). Sample absorbance 2.5 at 880 nm. Actinic light intensity $10^5$ erg $cm^{-2}$ $sec^{-1}$ (700–1100 nm).

the recorded absorption changes and to the fluorescence induced by measuring light [13]. To reduce the losses of light in low temperature experiments concentrated suspensions of chromatophores ($A_{880}$ = = 1 $mm^{-1}$) placed in a thin cuvette (2 mm optical path) were used. The freezing procedure took not less than 15 min for the liquid to dense phase transition to occur completely. As a result, the intensity of the measuring light beam at 80°K did not exceed 100 erg $cm^{-2}$ $sec^{-1}$.

The ESR measurements were made with an X-range spectrometer. The chromatophore suspension contained within a flat sample cell was placed in a Dewar flask which was held inside the microwave cavity. Small volume (0.008 ml) of the used sample cell allowed to minimize nonresonance absorption and to compare light-induced ESR signals at different temperatures.

## 3. Results

In the electron donor deficient medium at 297°K light-induced absorption changes of chromatophores (fig. 1) are characterized by the blue-shift at 800 nm ($\Delta A_{+795,-812}$) referring to the oxidation of bacteriochlorophyll in the reaction centres [14] and by the bleaching at 865 nm ($\Delta A_{-865}$). The observed changes saturate at the actinic light intensity of 5 × $10^3$ erg $cm^{-2}$ $sec^{-1}$; half-times ($t_{1/2}$) of their dark recovery being 250 msec.

In the presence of TMPD-$H_2$ (fig. 1A) the magnitude of $\Delta A_{+795,-812}$ absorption changes decreases whereas the red-shift in the absorption band of bulk bacteriochlorophyll B 880 ($A_{-865,+900}$) becomes pronounced. Under these conditions the saturating light intensity and the dark recovery half-time values for $\Delta A_{+795,-812}$ absorption changes were $10^4$ erg $cm^{-2}$ $sec^{-1}$ and 60 msec, respectively. Contrary to that, corresponding characteristics of $\Delta A_{-865,+900}$ absorption changes were quite different: 5 × $10^2$ erg $cm^{-2}$ $sec^{-1}$ and 10 sec.

These two types of spectral changes can be observed separately or together at different TMPD-$H_2$ concentrations and actinic light intensities.

Difference spectra of chromatophores at 80°K demonstrate no $\Delta A_{-865,900}$ shift, but only $\Delta A_{+795,-812}$ and $\Delta A_{-880}$ absorption changes which

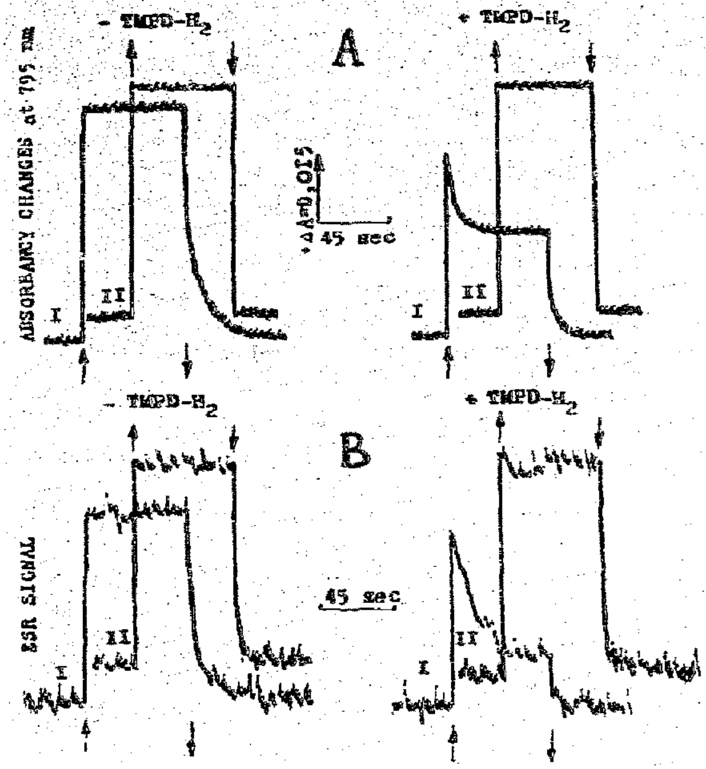

Fig. 2. Kinetic curves of light-induced optical absorbance changes at 795 nm (A) and those of ESR signal (B) in *R. rubrum* chromatophores. No addition – (−TMPD-$H_2$); in the presence of exogenous electron donor – (+TMPD-$H_2$). 1) at 297°K; 2) at 80°K. Sample composition and experimental conditions were the same as in fig. 1.

do not depend on the presence of TMPD-$H_2$ in the suspension (fig. 1). Although such low temperature spectra are similar in many respects to those obtained at 297°K in the absence of TMPD-$H_2$ their peculiar features are the pronounced broadening of the bleaching peak and 15 nm shift of its maximum position to longer wavelengths.

Instruments applied allowed to measure low temperature difference spectra in the 750–850 nm region with the second monochromator placed between the sample cuvette and the photomultiplier. It greatly improved the resolution of transmitted light from bacteriochlorophyll fluorescence. The fact that all results obtained by means of such technique proved to be in a good agreement with those discussed above (fig. 1) clearly indicates that no errors due to the fluorescence influenced the recorded difference spectra.

ESR measurements of the concentration of the photooxidized reaction centre bacteriochlorophyll confirms spectrophotometric data. Fig. 2 shows that amplitude and kinetic parameters of the optical absorbance changes centered at 795 nm and those of the ESR signal strictly correlate under different experimental conditions.

## 4. Discussion

In *R. rubrum* chromatophores supplied with TMPD-$H_2$ at 297°K actinic light illumination induces the electron flow accompanied by generation of the transmembrane potential [10]. It appears reasonable in this case to ascribe $\Delta A_{-865,+900}$ spectral changes mainly to the red-shift in the absorption band of the bacteriochlorophyll B 880 produced by the transmembrane electric field. In fact under these conditions the steady-state values of the $\Delta A_{-812,+795}$ absorption changes and ESR signal are rather small thus indicating to the relatively low concentration of photooxidized reaction centre bacteriochlorophyll. Hence there can be no substantial spectral shifts due to the small local field of the separated electric charges. The cessation of the electron flow under the exogenous electron donor deficiency or temperature lowering results in decrease of the transmembrane potential and therefore minimizes the corresponding spectral shift. On the other hand such treatments are favourable for the complete photooxidation of the reaction centre bacteriochlorophyll and, hence, for the development of the local electric fields. Spectral changes $\Delta A_{-865}$ ($\Delta A_{-880}$ at 80°K) observed under these conditions were usually attributed to photooxidative bleaching of the reaction centre pigment, absorbing around 870 nm, notwithstanding the fact that the major absorption band of the bulk bacteriochlorophyll was located at somewhat longer wavelengths in cells of the bacterium studied. The possible contribution to the $\Delta A_{-865}$ spectral changes of the B 880 band shift due to the action of local electric fields was neglected. One of us (V.D.S.), however, has recently shown that $\Delta A_{-865}$ changes are caused by the overlapping of the photooxidative bleaching of the reaction centre bacteriochlorophyll absorption band centered near 890 nm with red-shift in the absorption band of the bulk B 880 bacteriochlorophyll induced by the local electric field. Data presented in our paper are in a good agreement with these results. In fact, the spectral band of $\Delta A_{-865}$ changes is rather asymmetric, which can be the net result of an interference of the absorption decrease near 890 nm and absorption increase due to the red-shift of B 880 band in the same spectral region. Spectral position of the maximum of $\Delta A_{-865}$ changes corresponds to the maximal decrease of absorption, results from the B 880 band shift, and displaces to 880 nm as temperature lowers down to 80°K. The latter is consistent with well known data on the low temperature longwave shift of the B 880 absorbance band. Furthermore, anomalous broadening of the $\Delta A_{-865}$ band at 80°K is an additional sign of its complex structure.

## References

[1] R.K. Clayton, Molecular physics in photosynthesis (Blaisdell Publ. Co., N.Y., 1965).
[2] J. Amesz and W.J. Vredenberg, in: Currents in photosynthesis (Ad. Donkers, Rotterdam, 1966) p. 75.
[3] M.A. Cusanovich, R.C. Bartsch and M.D. Kamen, Biochem. Biophys. Acta 158 (1968) 397.
[4] R. Govindjee and C. Sybesma, Biochem. Biophys. Acta 223 (1970) 251.
[5] S. Okayama, T. Kakuno and T. Hokio, J. Biochem. 68 (1970) 19.
[6] N.V. Karapetyan, in: H. Metzner, Progress in Photosynthesis Research, Vol. 2, ed. H. Laupp, Jr. (Tubingen, 1969) p. 778.
[7] D. Fleischman and R. Clayton, Photochem. Photobiol. 8 (1968) 287.
[8] A.A. Kononenko, P.S. Venediktov, E.P. Lukashev and A.B. Rubin, Studia Biophys. D.D.R. 38 (1971) 9.
[9] E.L. Barsky and V.D. Samuilov, Biokhimiya USSR 37 (1972) 1005.
[10] P.I. Isaev, E.A. Liberman, V.D. Samuilov, V.P. Skulachev and L.M. Tsofina, Biochem. Biophys. Acta 216 (1970) 22.
[11] A.A. Kononenko, A.P. Andreytsev and A.B. Rubin, Biol. nauky USSR 8 (1967) 138.
[12] A.A. Kononenko, L.N. Grigorov, V.N. Verchoturov, A.P. Andreytsev and A.B. Rubin, Biol. nauky USSR 4 (1969) 128.
[13] A.Yu. Borisov, R.N. Ivanovsky and V.D. Samuilov, Biofizika USSR 14 (1969) 576.
[14] R.K. Clayton, Proc. Natl. Acad. Sci. U.S. 69 (1972) 44.