P_{430}, A POSSIBLE PRIMARY ELECTRON ACCEPTOR IN *RHODOSPIRILLUM RUBRUM*

Binah R. SILBERSTEIN and Zippora GROMET-ELHANAN
Department of Biochemistry, Weizmann Institute of Science, Rehovot, Israel

Received 27 March 1974

1. Introduction

A light-induced positive absorbance peak centered around 430 nm has been reported in cells and chromatophores of *Rhodospirillum rubrum* [1–3]. This 430 nm band was first attributed to the oxidation of reaction center bacteriochlorophyll [2,4] but it was later found to become saturated in whole cells at lower light intensities and to decay in the dark more rapidly than other bacteriochlorophyll bands [3]. It was also shown that this 430 nm band could not be due to photoreduction and dark reoxidation of cytochrome *b* since it was unaffected by 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide (HOQNO) that is known to block the reoxidation of cytochrome *b* [4,2], and it was much broader than a typical cytochrome band [5]. Arnold and Clayton have reported that this band could be observed even at 1°K [6] and therefore suggested that it might possibly be associated with a primary photochemical process.

Light induced increases in absorption around 430 nm have recently been observed also in other photosynthetic bacteria. Thus in *Rhodopseudomonas spheroides* an absorption change around 450 nm could be induced either photochemically or by strong chemical reduction [7]. The kinetics of this change were similar to those of the rise in P_{870} fluorescence and it was suggested that it could be due to a primary electron acceptor. A 424 nm change which was pronounced at −260 mV but was attenuated towards higher potentials, was found in *Chromatium* chromatophores [8,9]. In chloroplasts too an absorbance change at 430 nm has been reported and was attributed to the primary acceptor of photosystem I [10].

In the present communication we tested the effect of electron donors, acceptors and inhibitors on the light-induced 430 nm absorbance band (P_{430}) in *R.rubrum* chromatophores. Our results indicate that P_{430} does not correspond to the reaction center bacteriochlorophyll which serves as an electron donor, but seems to reflect the primary electron acceptor.

2. Materials and methods

The growth of *R. rubrum* cells and the isolation and storage of chromatophores were as previously described [11–13] Bacteriochlorophyll was determined using the absorbance coefficient in vivo given by Clayton [14]. Absorbance changes were followed in the Aminco Chance dual wavelength spectrophotometer equipped with an EMI 9592B S10 or Hamamatsu R316 S1 photomultiplier. For measurements from 410 to 600 nm, the S10 photomultiplier tube was blocked by a Corning CS 4-96 filter. Actinic illumination was provided by a 500 W slide projector (without its heat filter) and was filtered through a combination of Schott RG 645 and RG 9 filters and 8 cm of water. Between 600 and 830 nm the S1 photomultiplier was blocked by a Schott RG1 filter and an infrared reflecting mirror, λ of reflection > 850 nm in order to eliminate interference due to bulk bacteriochlorophyll fluorescence. Actinic illumination was filtered through a combination of Corning CS 4-96 and Schott OG 1 filters. The light intensity in both sets of experiments was 5×10^4 Erg × cm^{-2} × sec^{-1}.

The standard reaction mixture contained in a final

volume of 3 ml: Tricine–NaOH buffer, pH 7.5–20 mM $MgCl_2$–3.3 mM HOQNO–10 μM 1 μM carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) and 45 μg bacteriochlorophyll. Other conditions are stated in the text. Experiments under anaerobic conditions were carried out in Tunberg cuvettes that were evacuated in the dark for three minutes at 2 mm Hg.

3. Results

Under aerobic conditions the light minus dark difference spectrum of *R. rubrum* chromatophores features two positive peaks centered around 430 and 810 nm and two negative peaks at 600 and 790 nm (fig. 1). A similar difference spectrum was obtained under anaerobic conditions. When however, under anaerobic conditions an electron donor couple, 2,6-dichloroindophenol (DCIP) + excess ascorbate, was added the light minus dark bands at 810, 790 and 600 nm were markedly decreased whereas the 430 nm band was slightly increased (fig. 1). These results indicate that the 430 nm band is different than the other three bands which have been suggested to represent the photooxidation of reaction center bacteriochlorophyll.

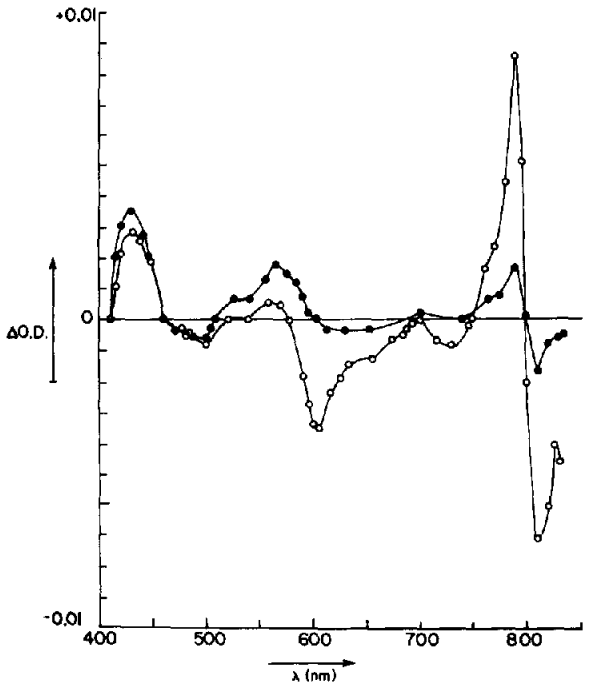


Fig. 1. Light minus dark difference spectrum of *R. Rubrum* chromatophores. ○, standard reaction mixture under aerobic conditions. ●, standard reaction mixture supplemented by 66 μM DCIP and 13 mM ascorbate under anaerobic conditions.

In an attempt to further characterize P_{430} it was found that this light-induced increase in absorbance was completely reversible in the dark (fig. 2a) and could be reproduced many times (not shown here). Since addition of dithionite in the dark was found to cause an increase in absorbance which was even larger than the light induced one, this P_{430} seems to represent a process of photoreduction and dark re-oxidation. This photoreduction at 430 nm is however different than the photoreduction of cytochrome *b* because it was observed in the presence of HOQNO [4]. In fact both the 'on' and 'off' kinetics were found to be identical in the absence and presence of HOQNO, and HOQNO was therefore added to all experiments. A pyrophosphate-induced increase in absorbance centered at 433 nm has recently been observed in *R. rubrum* chromatophores, and was reported to be inhibited by FCCP [15]. The light-induced increase in absorbance observed in fig. 2 could not be due to this energy dependent change since it was not affected by FCCP. In order to eliminate any complications due to energy dependent changes, FCCP was added to all experiments.

The rapid kinetics of P_{430} were not affected by depletion of oxygen or by the addition of the electron donor couple under air (fig. 2, A–C). When, however, the electron donor couple was added in the absence of oxygen, the light induced increase at 430 nm was still observable but the reversible decrease in the dark was drastically inhibited (fig. 2D). Such over-reducing conditions achieved by addition of an electron donor system in the absence of any added electron acceptor are also known to suppress photophosphorylation [16]. Since the inhibition of photophosphorylation could be relieved by the addition of electron acceptors [16] we examined their effect on P_{430} under over-reducing conditions (fig. 3). The rapid dark reoxidation reappeared upon the addition of benzyl viologen at 10^{-5} M (fig. 3A) and a similar effect was obtained in the presence of 10^{-4} M methyl viologen (the mid point potential of benzyl viologen

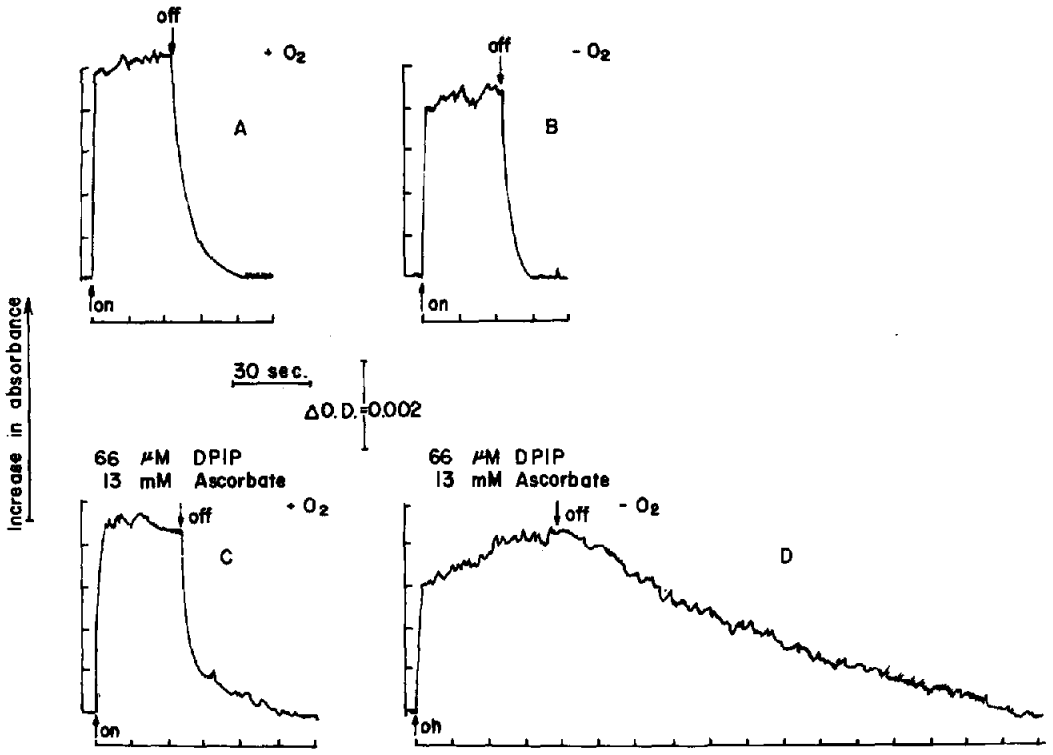


Fig. 2. Kinetics of P_{430} under aerobic and anaerobic conditions in the absence and presence of an electron donor system. Conditions as described in Materials and methods. P_{430} was measured at 430 vs. 460 nm because 460 nm was found to be an isosbestic point (fig. 1). Identical results were obtained when the reference wavelength was set at 410 nm.

is −320 mV and of methyl viologen −440 mV). NAD^+ enabled a rapid dark reoxidation of P_{430} but of only 20% of it (fig. 3B). In this case FCCP was omitted

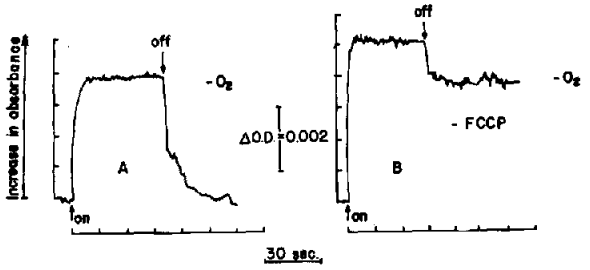


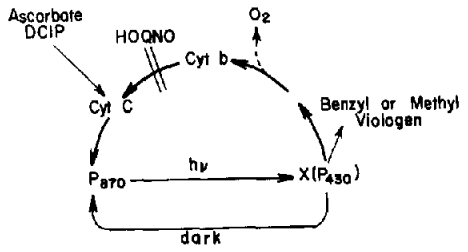
Fig. 3. Effect of benzyl viologen and NAD^+ on P_{430} under over reducing conditions. Experiments were carried out as in fig. 2D, A–with 10^{-5} M benzyl viologen, B with 3×10^{-4} M NAD^+ and without FCCP.

since it inhibits NAD^+ reduction [17,18]. Similar results were, however, obtained in the presence of NAD^+ and FCCP as well as in the presence of $NADP^+$ which does not act as an electron acceptor in washed chromatophores [17].

Although succinate was shown to be a better electron donor for NAD^+ reduction than ascorbate-DCIP in *R. rubrum* chromatophores [18,19] it did not behave as an electron donor to P_{430} because addition of succinate instead of ascorbate-DCIP under anaerobic conditions did not result in any inhibition of the dark reoxidation of P_{430}.

4. Discussion

An interpretation of the above reported results is proposed in the following scheme:



P_{430} which is reduced in the light and reoxidized in the dark is placed on the acceptor side of P_{870}. The dark reoxidation of reduced P_{430} (P^{-}_{430}) can proceed via the cyclic electron transport (through cytochromes *b* and *c*). Since the P_{430} change was found to react reversibly even at 1°K [6] it is suggested that it might represent the primary electron acceptor which can also be reoxidized via a direct dark return of electrons from P^{-}_{430} to oxidized P_{870} (P^{+}_{870}). Therefore, in presence HOQNO which blocks the cyclic electron transport [4], the photoreduction and dark reoxidation of P_{430} was not affected. When, however, in the presence of HOQNO an excess amount of electron donor was added so that P_{870} was kept in its reduced state, the reoxidation of P^{-}_{430} via both pathways was inhibited. These overreducing conditions were obtained under anaerobic conditions in the presence of DCIP + excess ascorbate (fig. 2D). A resumption of the reoxidation of P^{-}_{430} under these conditions will depend on the supply of a suitable electron acceptor. Oxygen seems to fulfill this requirement although the exact location of its electron accepting site is not known (dashed line in scheme). Under anaerobic conditions benzyl and methyl viologen were found to restore the rapid dark reoxidation of P^{-}_{430}. Since they are low potential acceptors their activity provides further evidence to our suggestion that P_{430} may be identified as the primary electron acceptor in *R. rubrum*.

The discrepancy between the effect of NAD^{+} and other electron acceptors on P_{430} and the observation that succinate does not serve as an electron donor may be explained by assuming that P_{430} is situated in the cyclic electron transport pathway which is not affected by succinate. It has been previously suggested [18] that the electron transport from succinate is mediated by a reaction center different from the one initiating the cyclic electron flow to which ATP formation is coupled.

Further investigation of P_{430} and its role in the electron transport system is now in progress.

References

[1] Smith, L. and Baltcheffsky, M. (1959) J. Biol. Chem. 234, 1575–1579.
[2] Duysens, L. N. M. (1957) in: Research in Photosynthesis (Brown, A. H., French, C. S., Livingston, R., Rabinowitch, E. I., Strehler, B. L. and Tolbert, N. E. eds.) 164–173, Interscience Publishers, Inc., New York.
[3] Vredenberg, W. J. and Duysens, L. N. M. (1964) Biochim. Biophys. Acta 79, 456–463.
[4] Nishimura, M. (1963) Biochim. Biophys. Acta 66, 17–21.
[5] Smith, L., Baltcheffsky, M. and Olson, J. M. (1960) J. Biol. Chem. 235, 213–218.
[6] Arnold, W. and Clayton, R. K. (1960) Proc. Natl. Acad. Sci. U.S. 46, 769–776.
[7] Clayton, R. K. and Straley, S. C. (1970) Biochem. Biophys. Res. Commun. 39, 769–776.
[8] Seibert, M., Dutton, P. L. and De Vault, D. (1971) Biochim. Biophys. Acta, 189–192.
[9] Seibert, M. and De Vault, D. (1971) Biochim. Biophys. Acta 253, 396–411.
[10] Hiyama, T. and Ke, B. (1971) Proc. Natl. Acad. Sci. U.S. 68, 1010–1013.
[11] Briller, S. and Gromet-Elhanan, Z. (1970) Biochim. Biophys. Acta 205, 263–272.
[12] Gromet-Elhanan, Z. (1970) Biochim. Biophys. Acta 223, 174–182.
[13] Gromet-Elhanan, Z. (1972) European J. Biochem. 25, 84–88.
[14] Clayton, R. K. (1963) in: Bacterial Photosynthesis (Gest, H., San Pietro, A., Vernon, L. P. eds.), pp. 495, Antioch Press, Yellow Springs, Ohio.
[15] Dutton, P. L. and Baltcheffsky, M. (1972) Biochim. Biophys. Acta 267, 172–178.
[16] Bose, S. K. and Gest, H. (1963) Proc. Natl. Acad. Sci, U.S. 49, 337–345.
[17] Keister, D. L. and Yike, N. J. (1967) Arch. Biochem. Biophys. 121, 415–422.
[18] Feldman, N. and Gromet-Elhanan, Z. (1971) in: Proceedings of the IInd International Congress on Photosynthesis Research (Forti, G., Avron, M. and Melandri, A. eds.) 1211–1220. Dr. W. Junk N.V. Publishers, The Hague.
[19] Keister, D. L. and Yike, N. J. (1969) Biochemistry 8, 167–173.
[20] Nozaki, M., Tagawa, K. and Arnon, D. I. (1961) Proc. Natl. Acad. Sci. U.S. 47, 1334–1340.