BBA Report

BBA 41163

A low potential photosystem in *Chromatium* D

MICHAEL SEIBERT, P. LESLIE DUTTON and DON DEVAULT
Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pa. 19104 (U.S.A.)

(Received October 12th, 1970)

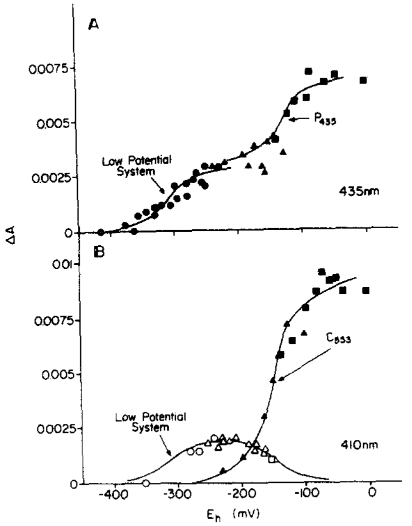
SUMMARY

A low potential light reaction in chromatophores of *Chromatium* D has been studied by means of oxidation–reduction potential titrations of laser-induced absorbance changes at 435 nm and 410 nm. This reaction, operating at potentials which are low enough to inactivate the P883 (P435) reaction center, has an apparent E_m of −145 mV and is attenuated as the potential is lowered with an E_m of −318 mV (n = 1). Its spectrum at −260 mV has a peak at 424 nm (compared with peaks at 423 nm and 432 nm for P435 at +415 mV), and its rise half-time appears to be around 75 nsec (compared with $<$ 50 nsec for P435). Although this system may represent either a new reaction center or a different form of P883, it is apparent that thermodynamically it is able to donate electrons to NAD$^+$.

Current evidence suggests that in *Chromatium* D energy absorbed by a single unit of bulk bacteriochlorophyll migrates to a single reaction center bacteriochlorophyll which first acts as the reductant for the primary electron acceptor and then serves as the oxidant for both cytochrome C555 (cyclic electron transport pathway) and cytochrome C553 (non-cyclic electron transport pathway)[1–5]. The reaction center bacteriochlorophyll has been identified by its light-induced absorbance changes at 883 nm (P883)[3,6,7] and at 435 nm (P435), the wavelength used in the present study. (Evidence indicating laser-induced P435 as a spectral component of the same reaction center bacteriochlorophyll as P883 is (a) it produces a similar oxidation–reduction midpoint potential (E_m) for the bacteriochlorophyll as produced by P883 (refs. 4, 5, 8) and (b) the kinetics of P435$^+$ reduction after a laser flash can be correlated with either the oxidation of cytochrome C553 or C555 (refs. 5 and 8) as has been demonstrated for P883 (ref. 2).) The E_m of the primary electron acceptor of the reaction center bacteriochlorophyll of this system ("P883 system") has been reported as −135 mV[4,9] or −160 mV[10] depending on the method of determination. At oxidation–reduction potentials (E_h) low enough to chemically reduce this primary electron acceptor, the P883 photosystem is no longer able to function since light-generated formation of oxidized bacteriochlorophyll is prevented by the absence of oxidized acceptor. This communication reports on a short-lived absorbance change induced by a 20-nsec, Q-switched ruby laser pulse (694 nm) at potentials low enough to virtually inactivate the P883 system.

The experiments were performed on chromatophores from *Chromatium* D prepared sonically as previously described[1,4]. They were suspended in buffer under strict anaerobic conditions in the cuvette of Dutton[4] which permits continuous stirring under an atmosphere of argon gas ($O_2 < 1$ ppm). Oxidation–reduction potential was assayed *via* a platinum electrode in conjunction with a standard calomel electrode. Redox mediators were added to act as oxidation–reduction potential equilibrium agents between the platinum electrode and the electron transport carriers in the chromatophore membrane.

Fig.1 shows the course of attenuation of the laser-induced absorbance changes at 435 nm (A) and 410 nm (B) as a function of lowering potential (the changes were not dependent on the nature and concentration of the mediating dyes). 435 nm is an isosbestic point for cytochrome C553 oxidation[1] and is used to assay P435; 410 nm is an isosbestic point for P435 (see Fig.2) and is used to assay cytochrome C553. There is no interference at



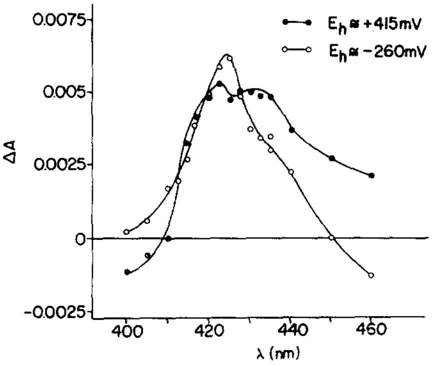


Fig.1. (A) Laser-induced absorbance changes as a function of oxidation–reduction potential at 435 nm for *Chromatium* D chromatophores suspended in 0.1 M Tris–HCl buffer (pH 7.4) under an atmosphere of argon gas. The absorbance changes were measured by subtracting the baseline from a fast-recovering component extrapolated back to the point where the laser was fired. The solid lines drawn through the points represent n = 1 curves. (B) Same as in (A) except that the monitoring wavelength was 410 nm. Solid symbols represent cytochrome C553 oxidation as measured by subtracting the baseline from the change approx. 14–16 μsec after the flash (C553 does not significantly recover in this time range). Open symbols represent the 410 nm spectral component of the fast-recovering low potential system measured by extrapolation back to the time of laser flash as in (A). The following mediators were present in (A) and (B): ●, ○, methyl viologen (50 μM), neutral red (50 μM), and benzyl viologen (50 μM); ▲, △, sodium anthraquinone-2-sulfonate (50 μM), sodium anthraquinone-2,6-disulfonate (50 μM), and 2-hydroxy-1,4-naphthaquinone (50 μM); ■, □, potassium indigodisulfonate (50 μM) and potassium indigotetrasulfonate (50 μM). Light path, 1 cm; bacteriochlorophyll concentration, 44 μM; temperature, 24 ± 2°.

Fig.2. Spectra of the two components described in Fig.1(A): ●—●, P435 (total ΔA) at +415 ± 5 mV, where cytochrome C553 and C555 interference is minimal (potassium ferricyanide); and ○—○, low potential system at −260 ± 5 mV (methyl viologen [50 μM], neutral red [50 μM], and benzyl viologen [50 μM]). A similar spectrum was obtained for the low potential component using sodium anthraquinone-2-sulfonate as a redox mediator and 860 nm light from a liquid dye laser as an excitation source. Light path, 1 cm; bacteriochlorophyll concentration, 39 μM.

either wavelength from cytochrome C555 since its oxidation is not evident at these low potentials[1,2,9]. Laser-induced absorbance changes at 435 nm diminish in two stages as the potential is chemically lowered. The first stage results from the inactivation of the P883 system as indicated by (a) the attenuation of P435 following a theoretical one-electron (n = 1) curve with an E_m of −127 mV (average of two experiments) and (b) the attenuation of cytochrome C553 in Fig.1(B) over essentially the same potential range (E_m = −133 mV, n = 1, average of two experiments). The E_m values obtained here compare well with those previously reported for the primary electron acceptor of the P883 system[4,9,10]. The second stage of attenuation of the laser-induced reactions at 435 nm has an E_m of −318 mV (average of two experiments). The spectrum generated by the laser at these low potentials is broad enough (see Fig.2) for the reaction to be observed at 410 nm, as seen in Fig.1(B) (open symbols) and explained in the figure legend. The course of attenuation with lowering potential at this wavelength is in close agreement with that observed at 435 nm, hence the E_m of the primary electron acceptor of the low potential photosystem would appear to be about −320 mV. Measurement at 410 nm also suggests that the unknown low potential component becomes evident with an estimated E_m of about −145 mV.

Fig.2 compares the laser-induced spectral changes of P435 and those of the low potential system. P435 (closed symbols) is characterized by a broad, split band[11] with maxima at 423 nm and 430–432 nm. The laser-induced absorbance changes of the low potential component (open symbols) produce a single absorbance maximum at 424 nm. Absorption increases at −260 mV were also observed at 547 nm and may represent another spectral component of the low potential system. Details in this spectral region, however, have not been determined.

Fig.3 shows the laser-induced kinetics of P435 and of the low potential photoreactive component; the rise half-time of the former is less than 50 nsec (the limit of the instrument) while that of the latter appears to be about 75 nsec. The reduction half-time of

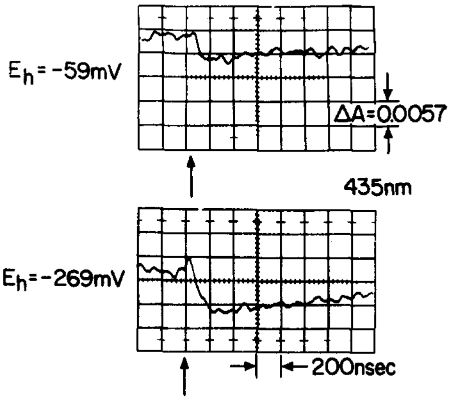


Fig.3. The kinetics of P435 and the low potential system. The upper trace indicates that the rise half-time of P435 is less than 50 nsec (at −59 mV with similar results at +410 mV), while the lower trace shows a 75-nsec rise time for the low potential component (−269 mV). The arrows indicate the time at which the laser was flashed. Wavelength, 435 nm; light path, 1 cm; bacteriochlorophyll concentration, 41 μM.

laser-generated P435$^+$ is about 1 μsec and matches the oxidation half-time of C553, while the recovery half-time of the low potential component is about 2.4 μsec during which no C553 oxidation is apparent. Fig.3 also illustrates the fact that in some experiments the maximum change in absorbance of P435 was less than that of the low potential component (this was not the case in Figs.1 and 2).

The present evidence indicates that the current ideas concerning the primary photochemical processes in bacterial photosynthesis must be re-evaluated. Since the nature of the low potential component is unknown, one must consider whether it is (a) a new reaction center–acceptor system operating at oxidation–reduction potentials lower than the P883–acceptor complex, (b) a different spectral form of P883 (created by a different charge environment when the −135 mV acceptor is chemically reduced) which can in the excited state donate electrons to the −318 mV acceptor when the −135 mV acceptor is not available, or (c) a dark reaction following an as yet undetected photo-induced reaction.

From a thermodynamic point of view it is possible that the acceptor of the low potential photosystem may serve for the ultimate reduction of NAD$^+$ ($E_{\mathrm{m}7}$ of NAD$^+$/NADH −320 mV[12]). Previous reports of light-induced absorbance changes (a) at 430 nm in *Rhodospirillum rubrum* in the presence of dithionite[11] and in the 730–790 nm region of the same bacterium poised at −220 mV[13] and (b) at 419 nm in *Rhodopseudomonas gelatinosa* poised at −350 mV before freezing to 77°K (ref. 4) suggest that the existence of a low potential photosystem may be a common feature of bacterial photosynthesis.

We are grateful to Dr. James McCray for setting up the liquid dye laser apparatus, to Drs. Britton Chance and Mitsuo Nishimura for discussion of the manuscript and to Miss Keiko Sera for her technical assistance. This research was supported by National Science Foundation Grant No. GB-6556 and by the Public Health Service Molecular Biology Training Grant No. 5-T01 GM-00694-10.

REFERENCES

1 M. Seibert and D. DeVault, *Biochim. Biophys. Acta*, 205 (1970) 220.
2 W.W. Parson and G.D. Case, *Biochim. Biophys. Acta*, 205 (1970) 232.
3 J.P. Thornber, *Biochemistry*, 9 (1970) 2688.
4 P.L. Dutton, *Biochim. Biophys. Acta*, 226 (1971) 63.
5 M. Seibert and D. DeVault, in preparation.
6 R.K. Clayton, in L.P. Vernon and G.R. Seely, *The Chlorophylls*, Academic Press, New York, 1966, p.609.
7 W.W. Parson, *Biochim. Biophys. Acta*, 153 (1968) 248.
8 M. Seibert, T. Kihara, P.L. Dutton and D. DeVault, *Abstr. Intern. Conf. Photosynth. Unit, Gatlinburg, Tenn., May 1970*, Abstr. F-3.
9 M.A. Cusanovich, R.G. Bartsch and M.D. Kamen, *Biochim. Biophys. Acta*, 153 (1968) 397.
10 W.A. Cramer, *Biochim. Biophys. Acta*, 189 (1969) 54.
11 W.W. Parson, *Biochim. Biophys. Acta*, 131 (1967) 154.
12 P.A. Loach, in R.C. Weast and G.L. Tuve, *Handbook of Biochemistry Selected Data for Molecular Biology*, The Chemical Rubber Company, Cleveland, 1968, p.J-27.
13 P.A. Loach, *Biochemistry*, 5 (1966) 592.