THE LIGHT-REACTION OF THE GREEN PHOTOSYNTHETIC BACTERIUM *CHLOROBIUM LIMICOLA* F. *THIOSULFATOPHILUM* AT CRYOGENIC TEMPERATURES

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1. Introduction

The photochemical reaction of the green sulfur photosynthetic bacteria (Chlorobiaceae) is clearly very different from that of the purple sulfur and non-sulfur bacteria (Chromatiaceae and Rhodospirillaceae). The latter have a quinone–iron complex, with an operating midpoint potential near −175 mV, as the 'primary acceptor' [1–3]. These organisms reduce pyridine nucleotides by energy-dependent reversed electron flow [3,4]. In contrast, the midpoint potential of the 'primary acceptor' in green bacteria has a substantially lower value, probably near −540 mV [5–7], and these organisms are capable of direct photoreduction of pyridine nucleotides [3,4,8]. However, the chemical identity of the 'primary acceptor' in green bacteria, such as *Chlorobium limicola* f. *thiosulfatophilum*, is not clear. In particular, there has been disagreement about the role of a component exhibiting electron paramagnetic resonance (EPR) absorption at g 1.90 and g 1.79 in the reduced form. The original report of this component suggested that it was a Rieske-type iron–sulfur center [9] with a midpoint potential of +160 mV at pH 7 [6]. Similar iron–sulfur centers are found in other photosynthetic systems [10–15], although the *Chl. limicola* f. *thiosulfatophilum* center was unusual in that it exhibited a pH-dependent midpoint potential near neutral pH ([6] c.f. [13]). In contrast, it was proposed [7] that the g 1.90 component is, in fact, a very electronegative component of the primary acceptor site and can be photoreduced at cryogenic temperatures. The data in [6] was explained [7] as arising from irreversible photochemistry occurring during manipulations of the frozen EPR samples prior to spectrometric analysis, pointing out that the E_m obtained [6] for the g 1.90 component was remarkably close to the E_m of *P*-840 the bacteriochlorophyll primary donor [16,17].

We have re-investigated the primary photochemistry of *Chl. limicola* f. *thiosulfatophilum* at cryogenic temperatures using membrane fragments (chromatophores) and a partially purified bacteriochlorophyll reaction center complex (unit membrane vesicles [5,18]). We find that the g 1.90 iron–sulfur center behaves as would be expected for a Rieske-type component rather than as a component involved in the primary photochemistry.

2. Methods

Chlorobium limicola f. *thiosulfatophilum*, strain 6230 (Tassajara) was grown semi-autotrophically as in [19] and harvested by centrifugation without alum. The bacteriochlorophyll *a* reaction center complex (partially-purified complex I) was prepared as in [5,19], except that frozen cells were used and 10 mM sodium dithionite replaced sodium ascorbate during preparation. The dithionite was removed from the concentrated complex I preparation by passage over a Sephadex G-10 column. Membrane fragments (chromatophores) were prepared from frozen cells by passing the cells (after thawing) through a French pressure cell twice at 20 000 p.s.i. and collected by centrifugation at 144 000 × g after low speed centrifugation to remove cell debris.

Redox potentiometry and the monitoring of light-induced ΔA following single-turnover flash-activation were performed as in [17,20]. For most experimental points, 8 flash-induced responses were averaged. EPR spectra were measured at 10 K using a Varian E109 spectrometer, equipped with a flowing helium cryostat, as in [21].

3. Results and discussion

It was suggested [7] that the g 1.90 center in 'chromatophores' from green sulfur bacteria could be easily photoreduced by room light during sample preparation, and thus, explained the midpoint potential near +200 mV reported [6] for the g 1.90 component at pH 7 as an artifact due to the chemical oxidation of P-840 when the potential was raised above +200 mV. If P-840 (E_m +250 mV [16,17]) were chemically oxidized prior to sample freezing, no photoreduction of the g 1.90 component by ambient light would be possible. While this explanation ignored the reported pH-dependence of the g 1.90 midpoint potential (−60 mV/pH, [6]) it might still be valid if P-840 had a pH-dependent midpoint. This appeared unlikely as all other reaction center bacteriochlorophylls are known to have pH-independent midpoint potentials [3], but it appeared important to check the effect of pH on the midpoint potential of P-840. Figure 1 shows that P-840 in complex I has the same midpoint potential at pH 8.5 (+250 mV) as it does at pH 6.8 [17]. The measured E_m for the g 1.90 component at pH 8.5 is +70 mV [6].

Using the measured midpoint potentials of P-840 and the g 1.90 component at pH 8.5, we have poised the redox potential of 'chromatophores' to provide 3 regimes. Poising a sample at E_h +320 mV should chemically oxidize both P-840 and the g 1.90 center, and eliminate all photochemistry. Poising the sample at +150 mV should chemically reduce P-840, but leave the g 1.90 center oxidized, while poising at an ambient potential of −60 mV should chemically reduce both P-840 and the g 1.90 component. If the g 1.90 component is indeed a component of the 'primary acceptor', photochemistry should be prevented by its prior chemical reduction. Figure 2 shows that this expectation, based on the rationale in [7] is not realized. The g 1.90 component was completely reduced at −60 mV (as would be expected for a component with E_m +70 mV) when the sample had been exposed to the usual manipulations in dim light. A g 1.94 component was also visible, which is presumably due to the iron–sulfur center with an E_m −25 mV reported [6]. Upon illumination the signal centered near g 2.003 grew significantly, and did not decrease when the actinic light was turned off. We attribute this signal to P-840$^+$ [5,7]. There was no increase in the g 1.90 signal amplitude during or after illumination.

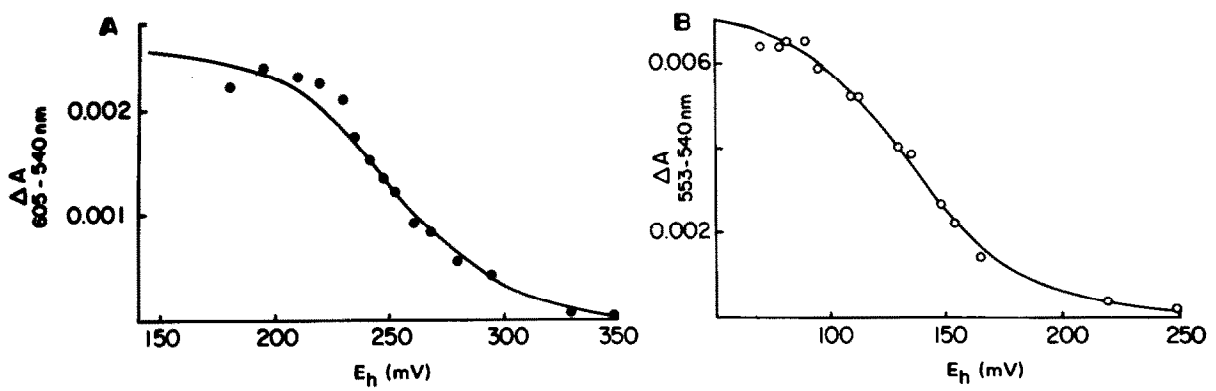

Fig.1. Redox titrations of the reaction-center bacteriochlorophyll (P-840) and cytochrome c_{553}. The reaction center complex (complex I) (A_{810} 2.6) was suspended in 20 mM glycylglycine, 100 mM KCl (pH 8.5) with 5 μM N-methyl phenazonium methosulfate, N-ethyl phenazonium ethosulfate, 2,3,5,6,- and N,N,N',N'-tetramethylphenylenediamine. The $\Delta A_{605-540}$ (A) and $\Delta A_{553-540}$ (B) after 8 flashes separated by 25 ms is plotted as a function of the ambient potential. The lines drawn are theoretical Nernst curves for the titration of P-840, E_m +250 mV (A) and cytochrome c_{553}, E_m +135 mV (B). These values compare with +250 mV and +165 mV measured at pH 6.8 [17].

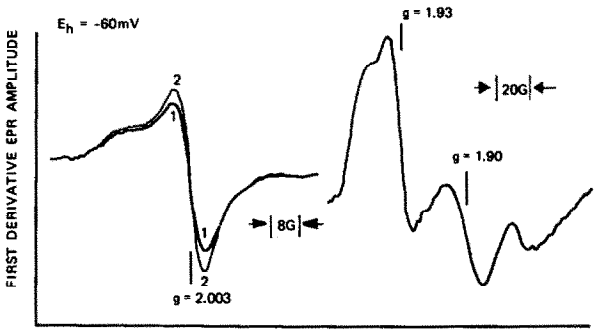

Fig.2. Iron–sulfur center redox states and *P*-840 photooxidation at an ambient redox potential of –60 mV. Chromatophores (equiv. 9.3 mM *Chlorobium* chlorophyll) were suspended in 50 mM Tris buffer (pH 8.5) with the redox mediators of fig.1 plus pyocyanine, all at 50 μM. Trace 1 (in the *g* 2.0 region) represents the sample in the dark prior to illumination while trace 2 was recorded during illumination. There was no further change in the signal when the light was turned off. EPR conditions: frequency, 9.057 GHz; modulation amplitude, 5 G in the *g* 2.0 region and 20 G in the *g* 1.93–1.90 region; microwave power, 2 mW in the *g* 2.0 region and 10 mW in the *g* 1.93–1.90 region; temperature, 10 K.

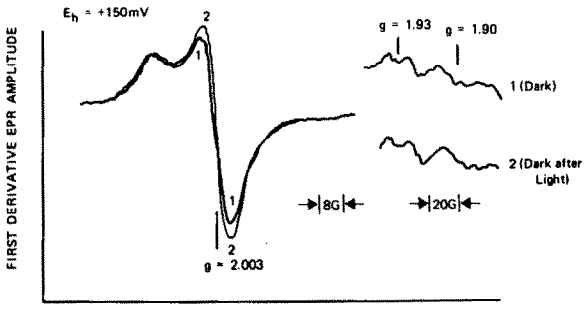

Fig.3. Iron–sulfur center redox states and *P*-840 photooxidation at an ambient potential of +150 mV. Reaction conditions as in fig.2.

The response of *P*-840 to light at potentials where the *g* 1.90 component was oxidized prior to illumination was identical to that observed at E_h –60 mV. Figure 3 shows that the light-induced increase at *g* 2.003 was irreversible at an ambient potential of +150 mV, just as it was at –60 mV. Furthermore, light minus dark difference spectra showed that the light-induced increase in the *g* 2.003 signal was equal in magnitude at the 2 ambient potentials. No absorbance at *g* 1.90 or *g* 1.94 could be discerned either before or after illumination at +150 mV. In contrast to observations made with samples prepared under these 2 regimes (–60 mV and +150 mV), no increase in the size of the *g* 2.003 signal was seen during or after illumination at an ambient potential of +320 mV.

These results disagree with those in [7], and are inconsistent with a role for the *g* 1.90 center in primary photochemistry at cryogenic temperatures. In agreement with [7] we did observe the irreversible appearance of an EPR signal at *g* 2.003 upon illumination of chromatophores at 10 K, but the magnitude of this signal, and its reversibility, were not affected by the prior chemical reduction of the *g* 1.90 component. The observation that no signal attributable to *P*-840$^+$ is generated in the light at an E_h +320 mV suggests that *P*-840 is chemically oxidized at this potential. Thus, the E_m for *P*-840 in chromatophores is probably similar to the value of +250 mV measured in complex I ([16,17] and fig.1). It would appear that the report of a higher value (+330 mV) using optical techniques was in error [22].

The observation that *P*-840 photooxidation in *Chl. limicola* f. *thiosulfatophilum* 'chromatophores' is irreversible at 10 K prompted us to re-investigate the reversibility of this reaction [5] in the partially-purified reaction-center preparation (complex I). Figure 4 shows that illumination (at 10 K) of complex I poised at an ambient potential (–370 mV) where photochemistry is possible, produced both irreversible and reversible EPR signals near *g* 2.0. Difference spectra revealed that both the reversible (light minus dark after light) and irreversible (dark after light minus dark) signals are centered near *g* 2.003 with peak-to-peak line widths near 9 G (see [5]). The amplitude of the irreversible signal was unchanged even when reaction-center photochemistry should have been eliminated by either the chemical pre-oxidation of *P*-840 (E_h +350 mV) or pre-reduction of the 'primary acceptor' (E_h –590 mV). In contrast, the reversible signal was essentially eliminated by these treatments (fig.4). Similar earlier results led us to conclude that only the reversible signal represented *P*-840$^+$ [5]. The species responsible for the irreversible signal has not been identified.

The primary light reaction of green sulfur bacteria, thus, appears to be reversible at 10 K in the

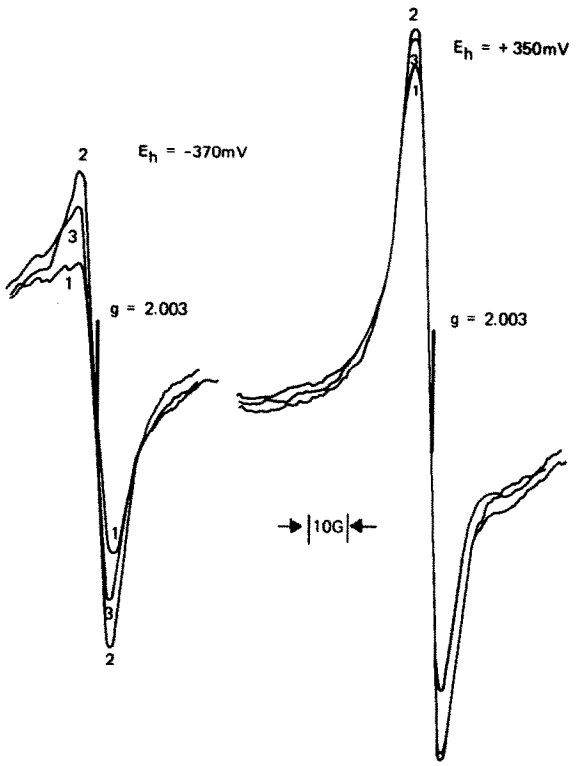

Fig.4. The effect of redox potential on the reversible and irreversible light-induced g 2.0 signals in complex I. The reaction center complex (complex I) (A_{810} 22.5) was suspended in 10 mM potassium buffer (pH 7.2), 25% sucrose with 10 μM *N,N,N',N'*-tetramethylphenylenediamine, benzyl viologen and methyl viologen. EPR conditions as in fig.2.

partially purified reaction-center complex (complex I), (fig.4 and [5]) but irreversible in 'chromatophores' (fig.2,3 and [7]). This may be because electrons can flow from the photoreduced 'primary acceptor' to a secondary acceptor in 'chromatophores', but not in complex I. Such secondary electron transfer would stabilize *P*-840$^+$ by eliminating the back reaction between *P*-840$^+$ and the photoreduced primary acceptor. Optical measurements at 5 K raise the possibility that the back reaction may become quite slow even in complex I when the temperature is lowered below 10 K [23].

We cannot yet identify either the primary or secondary electron acceptors of *Chl. limicola* f. *thiosulfatophilum*, although the data presented here appear to eliminate the possibility that the g 1.90 component fulfills either role. No light-induced reactions of this component were detected at cryogenic temperatures in either complex I or 'chromatophores', and the oxidation state of the g 1.90 component had no effect on the reversibility of *P*-840 photooxidation. We have no cogent explanation at this time for the discrepancy between our results and those in [7], who reported photoreduction of a g 1.90 component with $E_m < 500$ mV at 15 K. It may be possible that *Chl. limicola* f. *thiosulfatophilum* possesses 2 EPR-detectable centers at g 1.90 and that, due to some difference in technique, we detect only a Rieske-type center while only a low potential component of primary photochemistry was detected [7].

Clearly, much work remains to be done on the low potential acceptors of green bacteria. Unfortunately, this work is greatly hampered by the enormous complement of light-harvesting pigments [16] in these organisms, and the difficulty of obtaining purified preparations of the reaction-center complex I at high enough concentrations for EPR analysis. Nevertheless, it does seem clear that *Chl. limicola* f. *thiosulfatophilum* contains a Rieske-type iron–sulfur center. Recent mutant studies with plants have focused on the importance of such proteins in light-driven electron flow in these organisms [15], and the Rieske center in green bacteria may play an equivalent role.

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References

[1] Bolton, J. R. and Cost, K. (1973) Photochem. Photobiol. 18, 417–421.

[2] Prince, R. C. and Dutton, P. L. (1976) Arch. Biochem. Biophys. 172, 329–334.
[3] Prince, R. C. and Dutton, P. L. (1978) in: The Photosynthetic Bacteria (Clayton, R. K. and Sistrom, W. R. eds) pp. 439–453, Plenum Press.
[4] Knaff, D. B. (1978) in: The Photosynthetic Bacteria (Clayton, R. K. and Sistrom, W. R. eds) pp. 629–640, Plenum Press.
[5] Olson, J. M., Prince, R. C. and Brune, D. C. (1976) Brookhaven Symp. Biol. 28, 238–246.
[6] Knaff, D. B. and Malkin, R. (1976) Biochim. Biophys. Acta 430, 244–252.
[7] Jennings, J. V. and Evans, M. C. W. (1977) FEBS Lett. 75, 33–36.
[8] Evans, M. C. W. (1969) in: Progress in Photosynthesis Research (Metzner, H. ed) pp. 1474–1475, Laupp, Tübingen.
[9] Rieske, J. S., Hansen, R. E. and Zaugg, W. S. (1964) J. Biol. Chem. 239, 3017–3022.
[10] Dutton, P. L. and Leigh, J. S. (1973) Biochim. Biophys. Acta 314, 178–190.
[11] Evans, M. C. M., Lord, A. V. and Reeves, S. G. (1974) Biochem. J. 138, 177–183.
[12] Prince, R. C., Lindsay, J. G. and Dutton, P. L. (1975) FEBS Lett. 51, 108–111.
[13] Prince, R. C. and Dutton, P. L. (1976) FEBS Lett. 65, 117–119.
[14] Malkin, R. and Aparicio, P. J. (1975) Biochem. Biophys. Res. Commun. 63, 1157–1160.
[15] Malkin, R. and Posner, H. B. (1978) Biochim. Biophys. Acta 501, 552–554.
[16] Fowler, C. F., Nugent, N. A. and Fuller, R. C. (1971) Proc. Natl. Acad. Sci. USA 68, 2278–2282.
[17] Prince, R. C. and Olson, J. M. (1976) Biochim. Biophys. Acta 423, 357–362.
[18] Olson, J. M. and Thornber, J. P. (1979) in: Membrane Proteins in Energy Transduction (Capaldi, R. A. ed) Marcel Dekker, New York, in press.
[19] Olson, J. M., Philipson, K. D. and Sauer, K. (1973) Biochim. Biophys. Acta 292, 206–217.
[20] Dutton, P. L., Petty, K. M., Bonner, H. S. and Morse, S. D. (1975) Biochim. Biophys. Acta 387, 536–556.
[21] Prince, R. C., Leigh, J. S. and Dutton, P. L. (1976) Biochim. Biophys. Acta 440, 622–636.
[22] Knaff, D. B., Malkin, R. and Buchanan, B. B. (1973) Biochim. Biophys. Acta 325, 94–101.
[23] Whitten, W. B., Pearlstein, R. M. and Olson, J. M. (1979) submitted.