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SOME THERMODYNAMIC AND KINETIC PROPERTIES OF THE PRIMARY PHOTOCHEMICAL REACTANTS IN A COMPLEX FROM A GREEN PHOTOSYNTHETIC BACTERIUM

ROGER C. PRINCE and JOHN M. OLSON

Dept. of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, Pa. 19174 and Biology Department, Brookhaven National Laboratory, Upton, N.Y. 11973 (U.S.A.)

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Summary

We have examined the bacteriochlorophyll reaction-center complex of *Chlorobium limicola* f. *thiosulfatophilum*, strain Tassajara. Our results indicate that the midpoint potential of the primary electron donor bacteriochlorophyll of the reaction center is +250 mV at pH 6.8, while that of cytochrome *c*-553 is +165 mV. There are two cytochrome *c*-553 hemes per reaction center, and the light-induced oxidation of each is biphasic ($t_{1/2}$ of $< 5\,\mu s$ and $\approx 50\,\mu s$). We believe that this indicates a two state equilibrium with each cytochrome heme being either close to, or a little removed from, the reaction-center bacteriochlorophyll.

We have also titrated the primary electron acceptor of the reaction center. Its equilibrium midpoint potential at pH 6.8 is below -450 mV. This is very much lower than the previous estimate for green bacteria, and also substantially lower than values obtained for purple bacteria. Such a low-potential primary acceptor would be thermodynamically capable of direct reduction of NAD^+ via ferredoxin in a manner analagous to photosystem I in chloroplasts and blue-green algae.

Although a large volume of data has been obtained concerning the physical chemistry of light-induced electron flow in the photosynthetic bacteria, this has mainly been confined to the purple sulfur (Chromataceae) and purple non-sulfur (Rhodospirillaceae) groups (for a recent review see ref. 1). Relatively little is known about electron flow in the third group, the green sulfur bacteria (Chlorobiaceae). This has been partly due to technical difficulties, because while the photosynthetic units of the purple bacteria contain approximately 50 to 150 bacteriochlorophylls per reaction center, the Chlorobiaceae have in addition at least 1000 chlorobium chlorophylls as light-harvesting pigments [2].

Such a large background absorbance for each reaction center has made it difficult to monitor optical changes following single-turnover flash activation. However the forces which hold the vesicles of the green bacteria together appear to be qualitatively different from those involved in the purple bacteria and it is possible to use mild procedures, which do not involve detergents, to remove the chlorobium chlorophyll [2] to yield the "bacteriochlorophyll reaction-center complex". This complex is relatively large ($M_r > 1.5 \cdot 10^6$) and contains 80—100 bacteriochlorophylls per reaction center, together with carotene and several molecules of a *c*-type [2] and a *b*-type [3] cytochrome. It is analagous to a chromatophore from a purple bacterium in its pigment composition. Optical changes of this reaction-center complex from green bacteria can readily be monitored following flash excitation, and in this report we examine some kinetic and thermodynamic properties of the reaction-center complex.

Chlorobium limicola f. *thiosulfatophilum*, strain 6230 (Tassajara) was grown as previously described [4] and the bacteriochlorophyll reaction-center complex prepared [4] by a modification of the technique devised by Fowler et al. [2]. This involves breaking the bacteria in a French pressure cell, followed by density gradient centrifugation of the supernatant. Sodium ascorbate (10 mM) was present throughout the procedure. Redox potentiometry and the monitoring of light-induced changes following single-turnover flash-activation were performed in the apparatus previously described [5]. The actinic light was provided by a xenon flash lamp shielded with a Wratten 88A filter. Its width at half height was 6 μs, and it was approximately 80—85% saturating in intensity. When the averaging of flash induced responses was employed, sufficient time was left between activations to allow the system to recover completely.

Fig. 1 shows the response of the reaction-center bacteriochlorophyll *P*-840 (monitored at 605 vs. 540 nm) and cytochrome *c*-553 (monitored at 552 vs. 540 nm) to a train of 8 flashes of light. On a much faster time scale the absorbance decreases at 605 vs. 540 nm following each flash was almost as large as the total difference between the first and eighth flash, but on the time scale of Fig. 1, a large proportion of the *P*-840$^+$ is rapidly rereduced by cytochrome *c*-553. This is very similar to the situation in *Rhodopseudomonas sphaeroides* [5] where cytochrome c_2 rereduces the photo-oxidized reaction-center bacteriochlorophyll. The rereduction of the oxidized cytochrome *c*-553 was

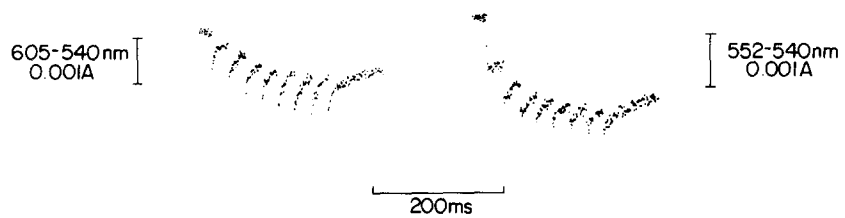

Fig. 1. Photo-oxidation of the reaction center bacteriochlorophyll and cytochrome *c*-553. The reaction-center complex ($A_{810\ nm} = 2.5$) was suspended in 25% sucrose, 10 mM sodium ascorbate, 10 mM sodium/potassium phosphate buffer pH 6.8. The figure shows the response of the reaction center *P*-840 and cytochrome *c*-553 to a train of 8 xenon flashes separated by 25 ms. Each trace is an average of 8 separate pulse trains, separated by 2 min.

complex, and because the preparation of the reaction-center complex required the presence of sodium ascorbate, it is probably this that rereduces the cytochrome. No changes ascribable to cytochrome *b* [3] could be detected under the conditions of Fig. 1.

Fig. 2 shows a spectrum of *P*-840 photooxidation in the 600 nm region. In order to resolve all the photo-oxidizable *P*-840 at potentials where some of the cytochrome *c*-553 was reduced prior to the flash, the extent of $P\text{-}840^+$ was measured as the total induced by a train of 8 flashes as in Fig. 1. Although the peak of the change was at 610 nm, for technical reasons we measured the change at 605 vs. 540 nm. Both 580 and 540 nm are apparently isosbestic wavelengths for the reaction-center complex. On the right of Fig. 2 is a redox titration of *P*-840. The midpoint potential at pH 6.8 was +250 mV, for a one-electron reaction.

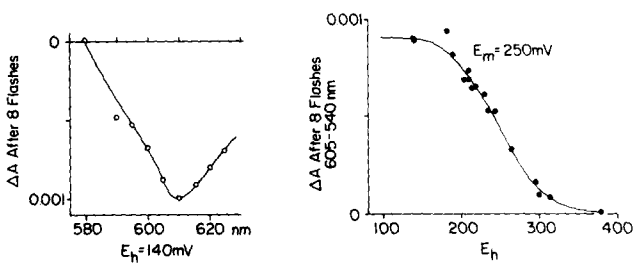

Fig. 2. Flash-induced spectrum and redox titration of the reaction-center bacteriochlorophyll. Conditions as for Fig. 1 except that only half the concentration of reaction center complex was present. 5 μM of each of *N,N,N*^{1},*N*^{1} and 2,3,5,6-tetramethyl phenylenediamine were present as redox mediators. For the spectrum, 580 nm was used as the isobestic wavelength. The line drawn through the titration points is a theoretical line for a one electron ($n = 1$) reaction.

Fig. 3 shows a spectrum of the α-band region of cytochrome *c*-553, and on the left is a redox titration of the first and second flash-induced cytochrome photo-oxidation. Fig. 1 showed that approximately half of the total flash-oxidizable cytochrome was oxidized on the first flash, even though almost all of the reaction center was oxidized. This suggested that there were two cytochrome *c*-553 hemes close to each reaction center. The theoretical lines drawn in Fig. 3 are those expected if two cytochrome *c*-553 hemes share one reaction center [6,7]. The fit to the data is very good. Interestingly, both *Chromatium vinosum* [6] and *Rps. sphaeroides* [7] also have two cytochromes *c* per reaction center.

Fig. 4 shows the kinetics of cytochrome *c*-553 photo-oxidation. At an ambient redox potential of +100 mV the rereduction of the cytochrome is relatively slow ($t_{1/2} \approx 200$ms) and the oxidation can be readily resolved into two phases of approximately equal extent, a fast phase ($t_{1/2} < 5$ μs) and slower phase ($t_{1/2} \approx 50$ μs). Dutton et al. [5] found a similar biphasic cytochrome c_2 photooxidation in *Rps. sphaeroides* and interpreted it as indicating a two state equilibrium of the cytochrome and a binding site on the reaction center. The fast phase was the oxidation of those hemes oriented favourably to the reaction center, while the slower phase was the oxidation of less favourable oriented molecules. We interpret our results with *C. limicola* f. *thiosulfatophilum* in a similar manner. It should be emphasized that the extent of photo

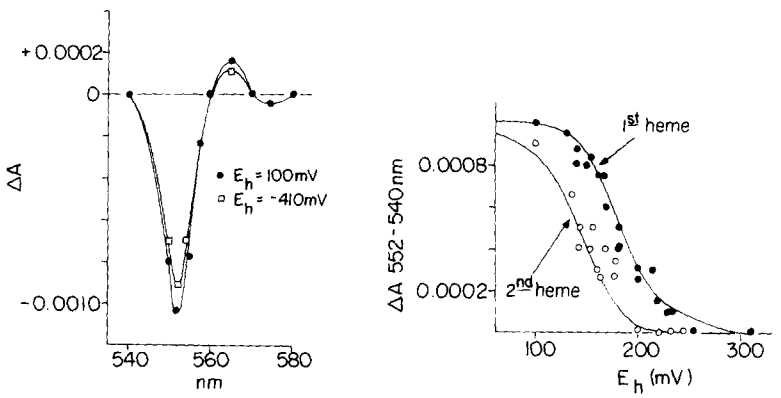

Fig. 3. Flash-induced spectrum and redox titration of cytochrome *c*-553. The conditions were the same as those of Fig. 1 with 7 μM each of *N,N,N*^{1},*N*^{1} and 2,3,5,6-tetramethyl phenylenediamine, *N*-methyl phenazine methosulfate and *N*-ethyl phenazine ethosulfate, plus methyl and benzyl viologen and pyocyanine for the low potential spectrum. The spectrum represents the extent of the first flash using 540 nm as an isosbestic wavelength. The titration of the first cytochrome was the extent of the first flash induced oxidation, the second cytochrome the sum of the second and third flash induced total oxidations. Each point is the average of 16 pulse trains separated by 1 min. The lines drawn through the points are those expected if two cytochrome hemes share one reaction center [6,7].

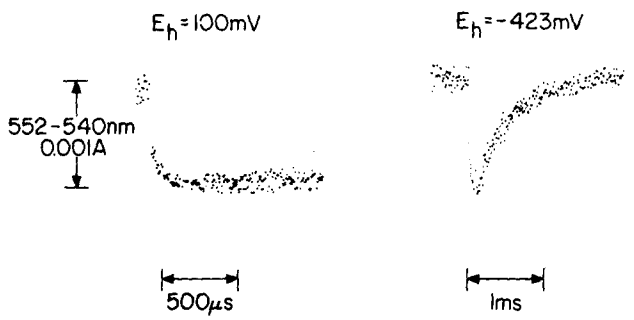

Fig. 4. The kinetics of light-induced cytochrome *c*-553 oxidation. The conditions were those of Fig. 3, except that only single flashes were used. Each trace is an average of 1024 single flashes, separated by 15 s, and the time constant in each case was 5 μs.

oxidation following a 20 ns ruby laser flash was indistinguishable from the extent caused by the 6 μs xenon flash, ruling out the possibility of the slow phase of cytochrome oxidation being extra turnovers of the reaction center caused by the xenon flash.

Our results for the midpoint potentials of *P*-840 and cytochrome *c*-553 are in excellent agreement with those of Fowler et al. [2] who examined a similar preparation from "*Chloropseudomonas ethylica*" (now known to be a symbiotic association of *Chlorobium limicola* and a colorless sulfate-reducing organism). They used continuous illumination and so could not resolve the two cytochrome *c*-553 hemes per reaction center. Our results are appreciably different form those of Knaff et al. [8] using "chromatophores" from the same strain of *C. limicola* f. thiosulfatophilum. Chromatophores still contain the light-harvesting chlorobium chlorophyll. Using continuous illumination they obtained values of + 330 mV for the midpoint potential of *P*-840 and +220 mV for the photo-oxidizable cytochrome *c* (α band at 551—552 nm).

These significant differences between "chromatophores" and bacteriochlorophyll reaction-center complexes deserve further study.

Knaff et al. [8] also attempted to measure the midpoint potential of the primary acceptor in their preparation, and obtained a value of - 130 mV. However, they took pains to point out that the value they obtained using continuous illumination could be erroneously high. We have investigated the midpoint potential of the primary acceptor in the reaction-center complex using single turnover flash activation, which removes many of the possibilities for error encountered when using continuous illumination. Our results are shown in Fig. 5. At pH 6.8, the lowest potential we could obtain using dithionite as a reductant was - 450 mV, and even at this low potential the photo-oxidation of cytochrome c-553 was unimpeded. At these low potentials the spectrum of the cytochrome (Fig. 3) and the kinetics of its oxidation (Fig. 4) were the same as those at higher potential, indicating that the same cytochrome was oxidized at all potentials. Our data at pH 6.8 indicated that the primary acceptor had a midpoint potential of substantially less than - 450 mV. Preliminary experiments at pH 9.6 indicated that the true value lies between - 500 mV and - 550 mV; further experiments to define this value more precisely are in progress.

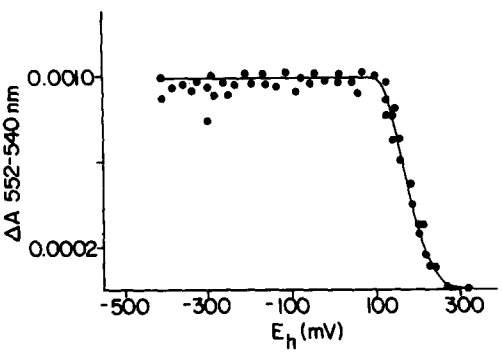

Fig. 5. The extent of light-induced cytochrome c-553 oxidation as a function of redox potential. Conditions as for Fig. 3 except that 10 μM concentrations of all the redox mediators were used. Each point is the average of 16 flashes at potentials above 0 mV, and of below this potential, because faster time constants were required at low potential to resolve the oxidation from the rapid rereduction.

This very low value for the primary acceptor of a green bacterium is substantially lower than any value encountered in the purple bacteria [1,9]. These latter organisms seem physiologically capable of reducing NAD^+ only by enery dependent reversed electron flow [1,9]. However, there is evidence that the green bacteria are capable of direct photoreduction of NAD^+ in a manner analagous to Photosystem I in chloroplasts and blue-green algae, albeit at a very slow rate [10—12]. This reaction can occur in the presence of inhibitors of reversed electron flow [12] and requires ferredoxin [10—12]. *C. limicola* f. *thiosulfatophilum* ferredoxin is unstable [10] and its midpoint potential is unknown. However, the reaction occurs when ferredoxin from *C. vinosum* is substituted [10—12] and this has a midpoint potential of - 490 mV [13]. A midpoint potential of the primary acceptor of *C. limicola* f. *thiosulfatophilum* of between - 500 and - 550 mV would be thermodynamically capable

of effective reduction of this ferredoxin, and is also in the same region as the values reported for the primary acceptor of chloroplast Photosystem I. (Estimates range from - 530 to - 580 mV [14,15]).

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