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ELECTRON SPIN RESONANCE CHARACTERIZATION OF *CHROMATIUM* D HEMES, NON-HEME IRONS AND THE COMPONENTS INVOLVED IN PRIMARY PHOTOCHEMISTRY

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SUMMARY

The combination of redox potentiometry with low temperature electron spin resonance (ESR) spectroscopy has led to further characterization of electron transfer components of *Chromatium* D. These include the readily buffer-soluble cytochromes c_{553} and c' and the high-potential iron–sulfur protein in the isolated state and associated with the chromatophore membrane. Buffer-insoluble cytochrome c_{553}, cytochrome c_{555}, bacteriochlorophyll and the primary electron acceptor have been characterized both in the chromatophore membrane and also in a sodium dodecylsulfate detergent-solubilized subchromatophore preparation. Two iron–sulfur proteins have been revealed which are present in the chromatophore membrane but are released on treatment with sodium dodecylsulfate. They have central g values at 1.90 and 1.94 and have estimated midpoint potentials at pH 7.4 ($E_{m7\cdot4}$) at +280 mV and −100 mV, respectively, when associated with the chromatophore.

In the membrane associated state the apparent E_m of cytochrome c' is approximately 200 mV more positive than the E_m values reported for the free state; this implies either that the reduced form of cytochrome c' binds to the membrane (or to a component therein) to a degree which is $>10^3$ times greater than that of the oxidized form or that the E_m shift results from membrane solvation. In the case of the high-potential iron–sulfur protein however, its E_m when associated with the chromatophore membrane is similar to that reported in the isolated state. The light-induced oxidation of the high-potential iron–sulfur protein at room temperature appears to be linked only to the oxidation of cytochrome c_{555}; it could serve as an electron pool in equilibrium with cytochrome c_{555} in the cyclic electron flow system.

The redox component defined in the reduced state by its $g_y=1.82$ and $g_x=1.62$ ESR spectrum satisfies the following criteria for its identification as the primary electron acceptor of P883. (a) The $E_{m7\cdot4}$ value of the $g=1.82$ component is -120 ± 25mV. (b) At −70 mV, where the $g=1.82$ component is mainly oxidized in the dark, brief illumination at low temperature which causes the irreversible oxidation of one cytochrome c_{553} heme, also induces the permanent reduction of the $g=1.82$ component; the extent of reduction after brief illumination, given by the $g=1.82$ signal height, is the same as that induced chemically at −270 mV showing it to be fully reduced by

Abbreviation: C.A.T., computer of average transients.

the receipt of a single electron. (c) At more positive potentials where cytochrome c_{553} is oxidized and is not involved in low-temperature reactions, the light-induced low-temperature kinetics of the $g = 1.82$ signal are reversible; the flash-induced $g = 1.82$ formation and subsequent dark decay are the same as those for the flash-induced P^+883 ($g = 2$) formation and dark decay. We suggest that until a full physical–chemical characterization is completed this $g = 1.82$ component be designated "photoredoxin".

INTRODUCTION

Extensive studies of the components involved in light-driven electron transport in the anaerobic photosynthetic bacterium, *Chromatium* D have yielded thermodynamic, kinetic and functional information necessary for an ultimate understanding of photosynthetic energy conservation. A great number of components have been resolved.

TABLE I

REDOX MIDPOINT POTENTIALS OF ELECTRON TRANSFER COMPONENTS IN *CHROMATIUM* D

	E_m (*mV*)	*pH*		*Ref.*
Reaction center bacteriochlorophyll	+490	7.5	Chromatophores	1
	+475	7.4	Chromatophores	2
	+490	7.7	Chromatophores	3
Cytochrome c_{555}	+320	7.5	Chromatophores	
	+325	7.0	Cholate solubilized	4
	+345	7.0	Sodium dodecyl sulfate solubilized (Fraction A)	4
Cytochrome c'*	+180	7.5	Chromatophores	1
	− 5	7.0	Buffer-soluble isolate	5
Cytochrome c_{553} (soluble)	+ 10	7.0	Buffer-soluble isolate	5
Cytochrome c_{553}** (insoluble)	+ 10	7.4	Chromatophores	2
	+ 10	7.7	Chromatophores	3
	+ 8	7.0	Cholate-solubilized isolate	4
	+ 8	7.0	Sodium dodecyl sulfate solubilized (Fraction A)	4
High-potential iron–sulfur protein (HiPIP)	+350	7.0	Buffer-soluble isolate	6
Primary electron acceptor	−135	7.5	Chromatophores	1
	−135	7.4	Chromatophores	2
	−129	7.4	Chromatophores	7
	−160	8.0	Chromatophores	8
	−134	7.7	Chromatophores	3
Secondary electron acceptor	− 90	7.7	Chromatophores	3

* Previously designated cytochrome *cc'*, *Rhodospirillum* heme protein (RHP) (see ref. 6).
** Also designated c_{552} (ref. 3) and $c_{552.5}$ (ref. 4).

Table I provides the oxidation–reduction midpoint potentials of most of the well-identified electron carriers. The reaction center bacteriochlorophyll P883 serves to directly oxidize both cytochrome c_{555} and cytochrome c_{553} (refs 1, 9, 10). Cytochrome c_{555} which is considered to be involved in cyclic electron transport[1,2,11], the system geared to the production of ATP, is a high-potential cytochrome. Cytochromes c_{553} exist in at least two forms, buffer-insoluble (*i.e.* bound) and buffer-soluble c_{553}. These "low-potential" cytochromes are considered to be involved in the coupling of substrate electrons into the photosystem[1,2,11]. Cytochrome c_{553} (bound) is kinetically faster than cytochrome c_{555}. Cytochrome c' has been suggested as part of the cyclic electron transfer system[1] but in reality very little is known about its function. The high-potential iron–sulfur protein (HiPIP) has been well characterized structurally, but almost nothing is known regarding its function in photosynthesis. The primary electron acceptor of P883 has been described in a preliminary report[12]. It has been identified as a redox component which in the reduced form exhibits an ESR absorption characterized by a center band at $g = 1.82$. A similar signal has been characterized in chromatophores and the reaction center protein from *Rhodopseudomonas spheroides* and also shown to have properties consistent with its identity as the primary electron acceptor[13,14].

This communication describes simple characteristics of the ESR properties of the cytochrome and non-heme components of *Chromatium* D; it presents some light-induced redox changes of the ESR-detectable redox carriers and further thermodynamic and kinetic evidence which substantiates the identification of the of the $g = 1.82$ component as the reduced form of the primary electron acceptor.

MATERIALS AND METHODS

Chromatium D was grown anaerobically in the light on the medium previously described[2] except that malate was replaced by succinate. Some work was done on autotrophically grown cells (see ref. 10); no major differences were evident. Chromatophores were prepared by the alumina-grinding method. The readily soluble high-potential iron–sulfur protein, cytochrome c' and cytochrome c_{553} were isolated according to the procedure of Bartsch and Kamen[5] and Bartsch[6]. Preparation of subchromatophore "Fraction A" was accomplished by the method of Thornber[15] which employed the detergent sodium dodecylsulphate; spectrophotometric analysis of the preparation was similar to that published by Thornber[15]. Bacteriochlorophyll concentration was measured at 590 nm using an extinction coefficient of 20 $mM^{-1} \cdot cm^{-1}$.

Redox potentiometry was performed under anaerobic conditions as previously described[2]. Redox dyes were employed to promote equilibrium between the platinum electrode and the membrane bound carriers. Samples taken anaerobically into 3-mm internal diameter quartz tubes were rapidly frozen to liquid-nitrogen temperatures and maintained in the dark.

Electron spin resonance was assayed with a Varian E3 ESR spectrometer. ESR spectra were obtained near liquid-helium temperatures. ESR difference spectra or the accumulation of the kinetics of flash-induced signals were obtained using a Varian C-1024 computer of average transients (C.A.T.).

Illumination was provided by a Unitron (8V; 5A) lamp or a 4-μs (full width at halfheight) xenon flash. Light was filtered through a 2-cm path of water and a Wratten 88A filter which transmitted wavelengths longer than 700 nm.

RESULTS

Soluble components

Fig. 1 shows the EPR spectra three buffer-soluble components isolated from the 144000×*g* supernatant of the alumina-ground cells of *Chromatium* D. The two cytochromes, designated cytochromes *c'* and c_{553}, are isolated in the oxidized state

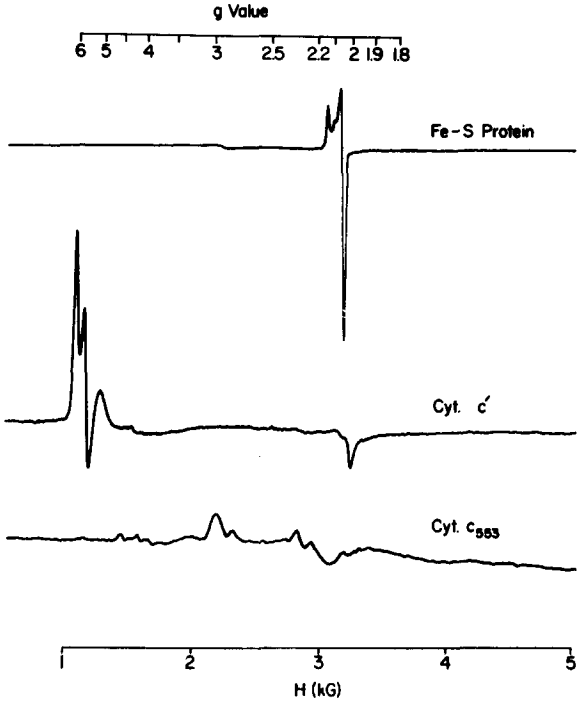

Fig. 1. ESR spectra of the buffer-soluble redox components. All three components are in their oxidized forms. It was necessary with the Fe–S protein to add a small amount of potassium ferricyanide since this was the high-potential iron–sulfur protein which was isolated in the reduced state. The samples were dissolved in 20 mM Tris–HCl buffer, pH 7.6. The sample temperature was approximately 15 °K with spectrometer microwave power setting of 1.0 mW.

and so are conveniently in the paramagnetic form. The Fe–S protein which has been designated "high-potential iron–sulfur protein"[6] is isolated in its reduced form; this compound also exhibits paramagnetism in the oxidized but not the reduced state, so a small amount of ferricyanide was added to the material before freezing. The spectrum of the iron–sulfur protein is similar to that described previously[16]. The EPR spectrum of the cytochrome *c'* shows it to be a high-spin heme with charateristic ESR signals in the $g=6$ region, while that of cytochrome c_{553} is a low-spin heme, similar to that of mammalian cytochrome *c* with a g_z signal at approximately $g=3$.

Components contained in the chromatophore and subchromatophore

(*a*) *High-potential iron–sulfur protein.* This component is clearly evident in chromatophores at +380 mV (compare Fig. 1 with Fig. 2). Appropriate for its known

+350 mV midpoint potential, the signal is insignificant at +200 mV. As may be expected from its ready solubility, the component is not present at significant levels in the subchromatophore Fraction A (Fig. 3).

(*b*) *Cytochrome c'*. Fig. 2 reveals what is probably cytochrome c' present in the chromatophore membrane as indicated by the peak at $g=6.0$ seen in the spectrum at +380 mV; at 200 mV the signal is very much diminished, implying that the cytochrome is mainly reduced at this potential. The midpoint potential can be estimated to be in the 250-mV region. This compares with the estimate of +180 mV by Cusanovich *et al.*[1] under similar conditions. The absence of the ferricytochrome c' signal at $g=6.0$ in the subchromatophore Fraction A (Fig. 3) is in accord with its known solubility and with Thornber's[15] analysis of this preparation.

(*c*) *Cytochrome* c_{555}. At potentials more positive than +380 mV, both chromatophores (Fig. 2) and subchromatophore Fraction A (Fig. 3) reveal four prominent signals in the $g=3$ region. In both preparations at 200 mV, two of the signals, those at approximately $g=3.1$ and $g=2.75$, are longer detectable. Between +380 and +200 mV the state of reduction of cytochrome c_{555} is known to be altered from mainly oxidized to essentially reduced (see Table I); thus either or both the $g=3.1$ and the $g=2.75$ signals can be ascribed to ferricytochrome c_{555}. The possibility does exist, however, that we are detecting the cytochrome $c_{553\ (550)}$ that was isolated by Cusanovich and Bartsch[19] for which they reported an $E_{m7.0}$ value of +350mV.

(*d*) *Cytrochrome* c_{553}. Of the four signals in the $g=3$ region shown in Fig. 2 and 3, the most dominant one is at $g=2.95$. This signal is still evident at +150 mV, but is very much diminished at −60 mV. This potential range over which the signal diminishes is consistent with the paramagnetism originating in ferricytochrome c_{553}. A way of verifying the identity of the $g=2.95$ signal as ferricytochrome c_{553} is afforded by the rapid and non-reversible oxidation of cytochrome c_{553} by light-induced P^+883 which can proceed rapidly at low temperatures[2,17] even at 4.2 °K[18]. A summary of the low-temperature reactions based on previous spectrophotometric studies[2,17] in *Chromatium* is given in Table II. Thus, brief illumination at low temperatures of material poised in the −10- to −70-mV redox potential region (where cytochrome c_{553} is mainly reduced and the primary electron acceptor mainly oxidized), should be expected to irreversibly generate the ESR spectrum of ferricytochrome c_{553}. This is shown in Fig. 4A. ESR spectra of chromatophores poised at −60 mV are shown in the dark before illumination (top), during illumination (center) and after illumination (bottom). The spectrum formed nonreversibly by illumination is that expected for ferricytochrome c_{553}. In addition to its g_z value of 2.95, we can also clearly identify the center band of absorption, the g_y signal, at approximately $g=2.3$. Fig. 4B shows that samples poised at potentials electronegative enough to essentially reduce the primary electron acceptor, no light-induced cytochrome c_{553} oxidation occurs because the prior reduced state of the primary electron acceptor prevents photooxidation of P883 which in turn prevents the oxidation of the cytochrome.

(*e*) *Other iron–sulfur proteins*. In chromatophores (Fig. 2), but not Fraction A (Fig. 3) two other signals are apparent with ESR characteristics consistent with tentative identification as reduced iron–sulfur proteins. A signal at approximately $g=1.90$ becomes evident over the 380- to 200-mV range; its midpoint potential can be estimated to be approximately 280 mV. At lower redox potentials a second signal at approximately $g=1.94$ becomes apparent between +95 and −260 mV. From the

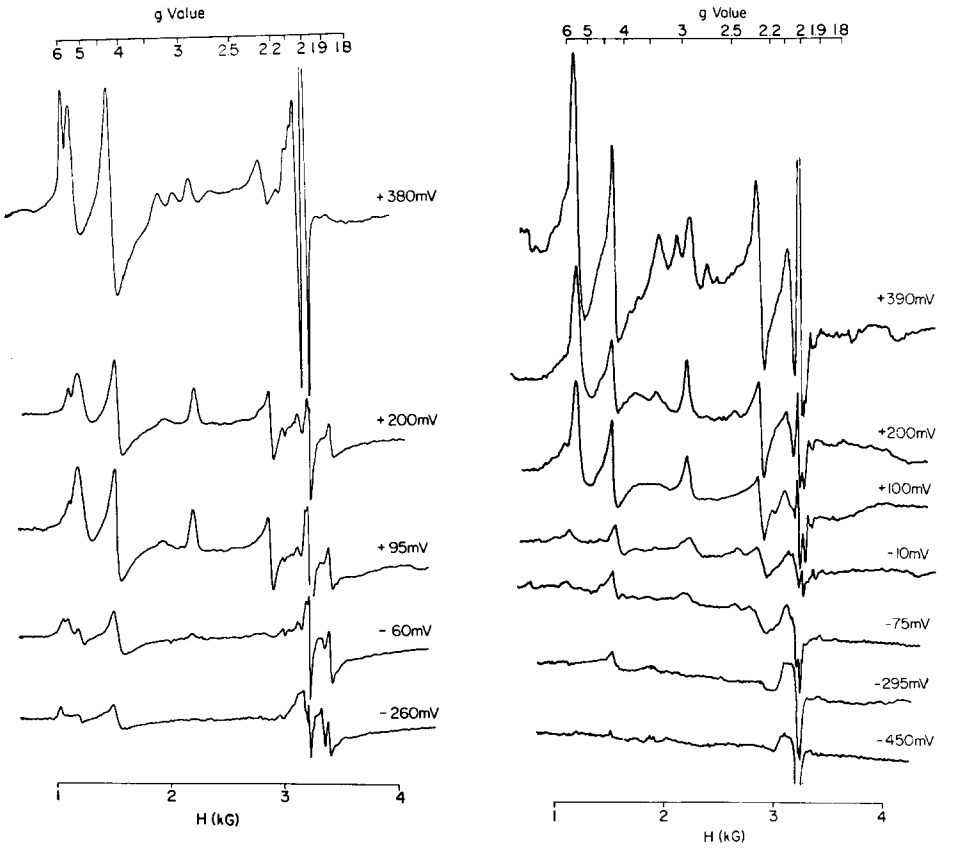

Fig. 2. Redox potential dependence of the ESR spectra of redox components associated with the chromatophore membrane. Chromatophores (bacteriochlorophyll concentration 3 mM) were suspended in 100 mM Tris–HCl, pH 7.4. The following redox mediators were present: diaminodurene 20 μM, phenazine methosulfate 20 μM, phenazine ethosulfate 20 μM, pyocyanine 10 μM, 2-hydroxy-1,4-naphthaquinone 20 μM, anthraquinone 2-sulfonate 20 μM. The sample temperature was adjusted to maximize the ferricytochrome c_{553} signal at $g=2.95$; this was approximately 15 °K. The spectrometer microwave power was 1 mW.

Fig. 3. Redox potential dependence of the ESR spectra of redox components associated with the detergent-solubilized subchromatophore Fraction A. The subchromatophores (bacteriochlorophyll concentration 0.55 mM) were measured under the same conditions as described for the chromatophores in Fig. 2 except that 20 μM methyl viologen was also present.

extent of its formation at -60 mV, its midpoint potential can be expected to be about -100 mV.

(*f*) *Unidentified signals.* Signals at $g=5.5$, $g=4.2$ and $g=3.4$, in both chromatophores and Fraction A subchromatophores remain unidentified. All appear to emanate from oxidized components, the reduction of which occurs mainly over the 200- to 0-mV range.

(*g*) *The primary electron acceptor.* Table II also shows the rationale from which we can predict the redox behavior of the primary electron acceptor, both in the dark and with illumination at low temperatures in *Chromatium* D. Thus at potentials

TABLE II

LOW-TEMPERATURE LIGHT-INDUCED REACTIONS IN *CHROMATIUM* D AT VARIOUS REDOX POTENTIALS

The symbol $\rightarrow$ // represents an electron transfer which is not feasible.

Potential region (mV)	*Redox states before freezing*	*Low-temperature reactions*
+100 to +400	P883 reduced c_{553} oxidized X oxidized	c_{553}^{3+} P+883 $\xrightarrow[<50\text{ ns}]{h\nu}$ X⁻ c_{553}^{3+} P883 $\xleftarrow[25\text{ms}]{\text{Dark}}$ X +
−10 to −70	P883 reduced c_{553} reduced X oxidized	c_{553}^{2+} P+883 $\xrightarrow[<50\text{ns}]{h\nu}$ X⁻ $c_{553}^{3+} \xrightarrow[\text{Dark}]{2\text{ ms}}$ P883 //← X⁻ //←
< −200	P883, c_{553} and X reduced	c_{553}^{2+} P883 $\xrightarrow{h\nu}$ // X⁻

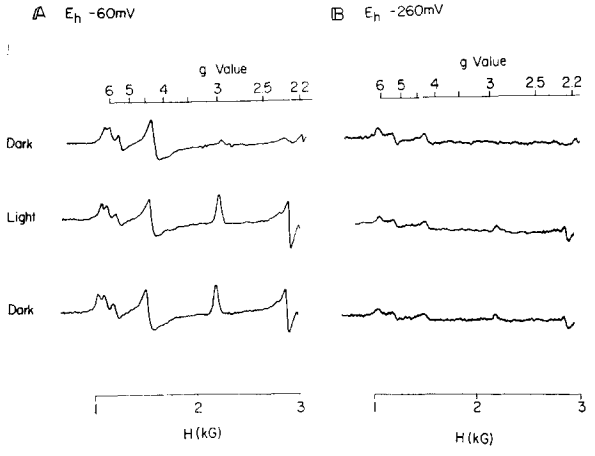

Fig. 4. Low-temperature light-induced oxidation of cytochrome c_{553} in chromatophores. The chromatophores (bacteriochlorophyll concentration 3 mM) were poised (A) at −60 mV and (B) at −260 mV. The samples were illuminated at 15 °K as indicated for approximately 1 s. Other conditions were as described in Fig. 2.

more negative than −200 mV, from its expected indirectly measured E_m value (see Table I), the primary electron acceptor will be essentially reduced, whereas above −60 mV it will be essentially oxidized. However, in the −60- to −10-mV potential region II, because of the irreversible oxidation of cytochrome c_{553}, brief illumination will cause the permanent reduction of the primary electron acceptor. This contrasts with the illumination of material poised in the potential region above +100 mV

(cytochrome c_{553} chemically oxidized) where the primary acceptor will be reversibly reduced by illumination. We have previously presented some evidence that the primary electron acceptor in the reduced form exhibits an ESR signal centered at $g=1.82$ (see refs 12–14). Fig. 5 shows the redox potential dependency of the $g=1.82$ signal in dark subchromatophore Fraction A; the measured $E_{m7\cdot4}$ value is -120 ± 25 mV. Fig. 6A shows typical ESR spectra of the Fraction A subchromatophore preparation poised at -70 mV (left), and at -270 mV (right). At -70 mV after brief illumination a signal centered at $g=1.82$ with a g_x band at $g=1.62$ is non-reversibly formed.

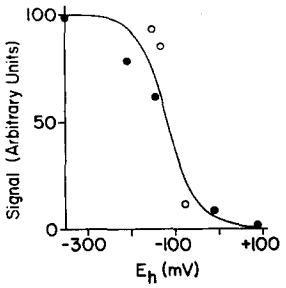

Fig. 5. The redox potential dependence of the course of oxidation–reduction of the $g=1.82$ component in subchromatophore Fraction A. The subchromatophores (bacteriochlorophyll concentration 1.0 mM) were in 100 mM MOPS buffer at pH 7.4, poised at various potentials in the dark before freezing. 50 μM phenazine ethosulfate, 100 μM pyocyanine, 150 μM 2-hydroxy-1,4-naphthaquinone, 100 μM phenosaphranine, and 50 μM benzyl viologen, were present as mediators. The sample temperature was 8 °K (which is as low as the equipment permitted) with a 20-mW spectrometer microwave power to maximize the $g=1.82$ component signals. The reduction was measured by the extent of the $g=1.82$ signal. The line drawn through the points is theoretical for a one-electron redox couple with an E_m -120 mV. Samples were taken in both an oxidative (○) and reductive (●) phase of the titration.

Fig. 6B shows a light *minus* dark difference spectrum of this change. In Fig. 6A at -270 mV, where the primary electron acceptor will be expected to be 99% reduced (see Fig. 5) this signal is already formed in the dark and illumination has no significant effect on the signal. The signal amplitude chemically induced by lowering the potential to -270 mV is the same as that induced by illumination at -70 mV. This suggests that on brief illumination at these temperatures, the $g=1.82$ component becomes fully reduced.

At the higher potentials where cytochrome c_{553} is already oxidized, the flash-induced reversible reduction and dark re-oxidation of the primary electron acceptor and the oxidation and re-reduction of P883 should have the same kinetics. Fig. 7 shows that the $g=1.82$ component satisfies this criterion. A similar exact relationship between the $g=1.82$ component and P883 has been reported for the isolated reaction center protein from *Rps. spheroides*[14].

These results strongly support the identification of the $g=1.82$ ($g_x=1.62$) component with the primary electron acceptor of reaction center P883. Further, the full reduction of the $g=1.82$ component after brief illumination at -60 mV where the only other net light-induced redox change is in the oxidation of one heme of cytochrome c_{553} (ref. 2) is consistent with the electron capacity of the primary electron acceptor being unity. Previous work on the laser-activated redox reactions of P883 measured

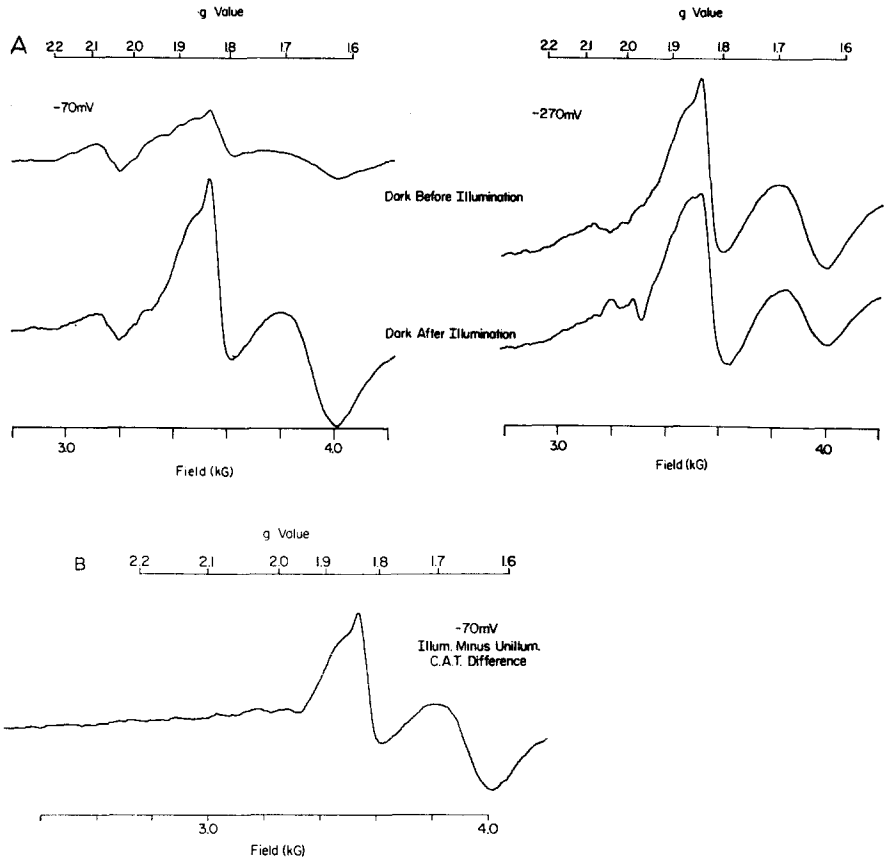

Fig. 6. Low-temperature light-induced reduction of the $g=1.82$ component in subchromatophore Fraction A at -60 mV and comparison with the extent of $g=1.82$ component observed chemically reduced at -270 mV. A. The subchromatophores (bacteriochlorophyll concentration 2.2 μM) in 100 mM Tris–HCl buffer, pH 7.4, were poised at the potentials indicated. 50 μM concentrations of phenazine methosulfate, pyocyanine, 2-hydroxy-1,4-naphthaquinone and anthraquinone 2-sulfonate were present as mediators. Other conditions were as described in Fig. 5. B. C.A.T. difference spectrum of material poised at -70 mV before and after brief illumination.

optically[17] or by ESR[12], and on the laser-induced formation of bacteriochlorophyll triplet state[20] at low temperatures has shown this to be the case.

Some light-induced reactions at room temperature. In the early work with *Chromatium* chromatophores Cusanovich *et al.*[1] studied the continuous light-induced reactions of material poised at known redox potentials before illumination. Although there was the risk that the mediating dyes would interfere with these reactions, they provided the first indications that cytochrome c_{553} oxidation occurred in preference to that of cytochrome c_{555}. Fig. 8 shows similar experiments on *Chromatium* D chromatophores and Fraction A poised at potentials (a) where cytochrome c_{555} is reduced and (b) where both cytochrome c_{555} and c_{553} are reduced before illumination. In samples poised such that of the two cytochromes only cytochrome c_{555} is reduced prior to room-temperature illumination, the signals at $g=3.1$ and $g=2.75$ appear correspon-

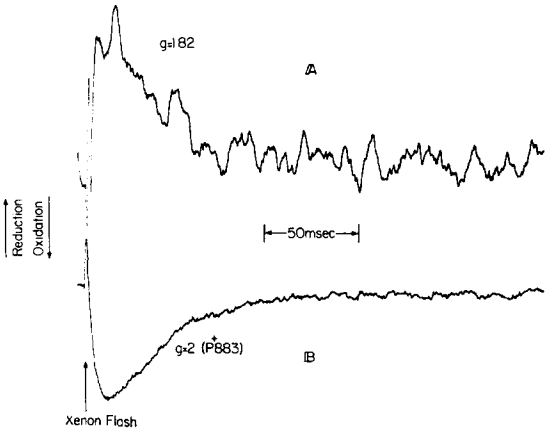

Fig 7. The kinetics of xenon flash-induced ESR changes at low temperature in subchromatophore Fraction A poised at 200 mV. The subchromatophore (bacteriochlorophyll concentration 1.5 mM) in 100 mM Tris–HCl buffer, pH 7.4, were poised at +200 mV using 40 μM concentrations of diaminodurene, phenazine methosulfate and phenazine ethosulfate as mediators. At $g=1.82$ (A) the kinetics of the change after 100 flashes were accumulated on the C.A.T.; at $g=2.0$ (B) the kinetics of the change after 10 flashes were accumulated. The instrumental rise time was approximately 5 ms. Other conditions are as described in Fig. 5.

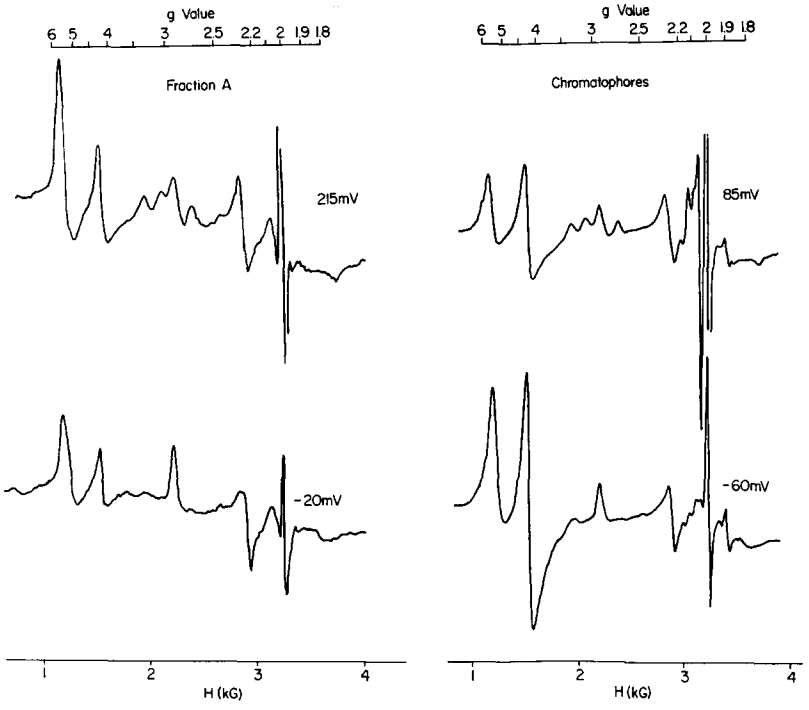

Fig. 8. The effect of illumination at room temperature of chromatophore and subchromatophore Fraction A. The preparation under conditions described in Figs 2 and 3 were poised at known potentials in the dark, illuminated for 10–15 s and frozen in liquid nitrogen and maintained in the dark. The potentials given in the figure refer to the values at the point of illumination; thus in the upper traces cytochrome c_{555} and high-potential iron–sulfur protein were reduced and cytochrome c_{553} oxidized before illumination, and in the lower traces cytochrome c_{553} was also mainly reduced before illumination.

ding to the previous tentative identification with oxidized cytochrome(s) c_{555}*. It is also clear in the chromatophores from the signal in the $g=2.04$ region, that the high-potential iron–sulfur protein becomes oxidized during the period of illumination.

At potentials negative enough to reduce cytochrome c_{553}, the only signal in the $g=3$ region to appear after illumination in that of 2.95 which corresponds to ferricytochrome c_{553}. Signals attributable to ferricytochrome c_{555} or oxidized high-potential iron–sulfur protein are absent after illumination. The results (a) agree with the experiments of Cusanovich *et al.*[1], (b) and further support for our above assignments of the EPR signals to cytochromes c_{555} and c_{553}, and (c) indicate that the high-potential iron–sulfur protein oxidation is linked only to that of cytochrome c_{555}.

DISCUSSION

Fig. 9 summarizes the redox potentiometric and ESR characteristics of the resolved electron transfer components as they exist in the chromatophore membrane of *Chromatium* D; they are shown in relation to the rapid electron transfer events closely

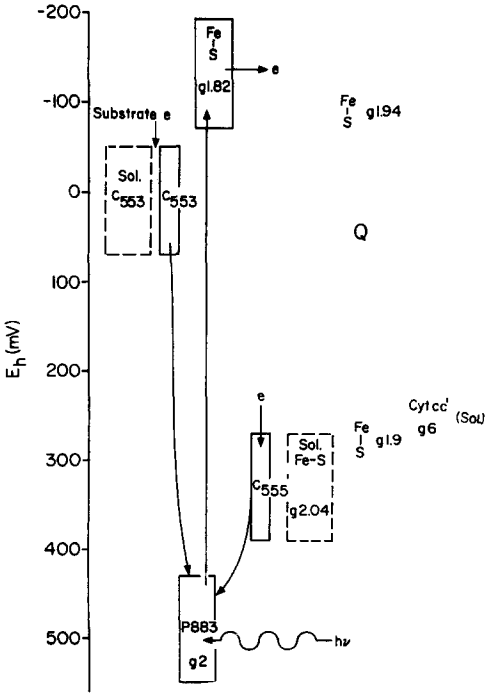

Fig. 9. Scheme showing the oxidation–reduction potential and ESR properties of the components associated with the *Chromatium* D chromatophore membrane. The blocks represent the potential ranges over which the components become 9–91% oxidized or reduced. The dashed blocks are of the buffer-soluble components. Components for which insufficient data is available to describe complete redox properties are shown without blocks.

*If, as we considered in Section (d), one of these bands is due to ferricytochrome $c_{553\,(550)}$ then it may be suggested that this cytochrome is active in electron transport.

associated with the reaction center system (see refs 2,3,9,10). We can assign the $g=2.95$ signal with the bound (buffer-insoluble) ferricytochrome c_{553} and either or both $g=2.75$ and $g=3.1$ signals with ferricytochrome c_{555}.

Apart from the assignments of ESR spectral bands to known electron carriers, two new components have been revealed; from their ESR spectra in their reduced forms we can tentatively identify them with iron–sulfur proteins. One has a central g value at 1.94 and approximate E_m −100mV; the other has a central g value at 1.90 and approximate E_m +280 mV. It may be of relevance that the former iron-sulfur protein has a similar E_m value to that of the secondary electron acceptor, the chemical identity of which is unknown (see Table I and ref. 3). The latter iron–sulfur protein is similar both in ESR spectrum and E_m to the mitochondrial iron–sulfur protein associated with the cytochrome b–c_1 region of the respiratory chain[21]. Both are present in the chromatophore membrane but neither is detectable in the detergent solubilized subchromatophore Fraction A.

It is interesting to note cytochrome c' when associated with the membrane assumes a measured E_m value some 200 mV more positive than the values reported for that in an aqueous buffer medium (see Table I). In this respect we confirm the finding of Cusanovich *et al.*[1]. Although this could be a membrane–solvent effect, it may also arise from a preferential binding of the ferrocytochrome c' to the membrane. A differential binding affinity of reduced ferrocytochrome c' which is $>10^3$ times greater than for ferricytochrome c, could cause a shift in apparent midpoint potential of 200 mV. The E_m of high-potential iron–sulfur protein, on the other hand, appears to be similar whether associated with the membrane or in the free state. The role of both these components remains obscure. The experiments shown in Fig. 8 however indicate that the high-potential iron–sulfur protein may be in efficient contact with cytochrome c_{555}; it could therefore serve as a redox pool at the electro-positive end of the energy conservation site in the cyclic electron transfer system.

We have extended our work with the $g=1.82$ component. Evidence that it is the first electron carrier to accept an electron from the reaction center P883 is as follows: (a) It is apparent in the detergent solubilized subchromatophore Fraction A. (b) Its E_m value is close to that expected for the primary electron acceptor. (c) In material poised at −60 mV, brief illumination near liquid-helium temperatures results in its complete and irreversible reduction. This is expected for the primary electron acceptor because of the irreversible oxidation of cytochrome c_{553} which occurs under these conditions. As already discussed, the fact that full reduction of the $g=1.82$ component occurs in a reaction sequence in which the only other net oxidation–reduction reaction is the oxidation of a single cytochrome c_{553} heme, is consistent with the electron capacity of the primary electron acceptor being unity (as expected from refs 2, 12, 17, 20). (d) At more positive potentials where cytochrome c_{553} is chemically oxidized and hence does not interfere with the return of the electron from the photoreduced primary acceptor to P^+883, the kinetics of flash-induced reduction and subsequent dark reoxidation of the $g=1.82$ component at low temperatures are identical to those of flash-induced P883 oxidation and dark re-reduction.

On account of the properties of the $g=1.82$ component and its possible identification as an iron–sulfur protein (see refs 14, 22, 23) we suggest that this component be designated "photoredoxin".

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