The Primary Electron Acceptor in Photosynthesis

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Summary

ESR studies on light induced reactions in Chromatium D at liquid helium temperatures reveals that the primary electron acceptor of reaction center bacteriochlorophyll has a signal at approximately g 1.82. Hence, the primary electron acceptor is probably an iron-sulphur protein. Using the same approach with spinach chloroplasts, a signal which is typical of a reduced iron-sulphur protein of plants is formed after a brief period of illumination.

Introduction

The photosynthetic bacterium Chromatium D displays light induced electron transfer reactions at liquid helium temperatures (1,2). Three components are involved: the reaction center bacteriochlorophyll P883, the P883 primary electron acceptor X, and a P883 primary electron donor, cytochrome $\underline{c}_{553}$. Thermodynamic and kinetic properties of the interactions of these components at low temperatures have been revealed by spectrophotometry (1,3,4) and by electron spin resonance (ESR) techniques (2,5). A summary of the reactions is presented in the accompanying manuscript (2).

Chromatophores or subchromatophores [Fraction A of Thornber (6)] can be poised at oxidation-reduction potentials before freezing such that P883 and cytochrome $\underline{c}_{553}$ are chemically reduced and X is oxidized (region II; see refs. 2,3). After brief illumination at low temperatures, cytochrome $\underline{c}_{553}$ is

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irreversibly oxidized and X is irreversibly reduced. P883, in donating an electron to X and then accepting one from the cytochrome, undergoes no net change of oxidation-reduction state. Hence the illuminated minus unilluminated ESR difference spectra will show only the net changes: oxidized cytochrome $\underline{c}_{553}$ and reduced X. The paramagnetic properties of oxidized $\underline{c}$-type cytochromes are well known; horse heart cytochrome $\underline{c}$ for example has readily detectable ESR components at g 3.06 and 2.24 (7).

Green plant chloroplast preparations at low temperatures are known to display light induced electron transfer reactions which result in the irreversible oxidation of cytochrome (8-10). Although they are not as well characterized as in the case of Chromatium D we have undertaken preliminary, parallel experiments with spinach chloroplasts.

Materials and Methods

The experimental procedure for Chromatium D, unless otherwise stated, was as described in the accompanying manuscript (2). Chloroplasts were isolated from spinach using a blender followed by differential centrifugation. ESR difference spectra were obtained from single scans using a Varian C-1024 Time Averaging Computer (CAT).

Results

The ESR spectra of Chromatium D subchromatophores (Thornber Fraction A) poised at -60 mV (region II) before freezing are shown in Figure 1A. The "dark" spectrum is of previously unilluminated subchromatophores. The "dark after illumination" spectrum is the same sample after illumination for a few seconds at liquid helium temperatures. Significant light induced changes are immediately discernable at approximate g values 2.9, 2.25 and 1.82. The light minus dark difference spectrum (Figure 1C) reveals the changes more clearly. Control difference spectra are presented in Figure 1B and D.†

† Differences are sometimes apparent as observed in B at g 2 but this arises from slight errors in the superpositions of the spectra being subtracted and only occurs at points of sharp spectral transitions.

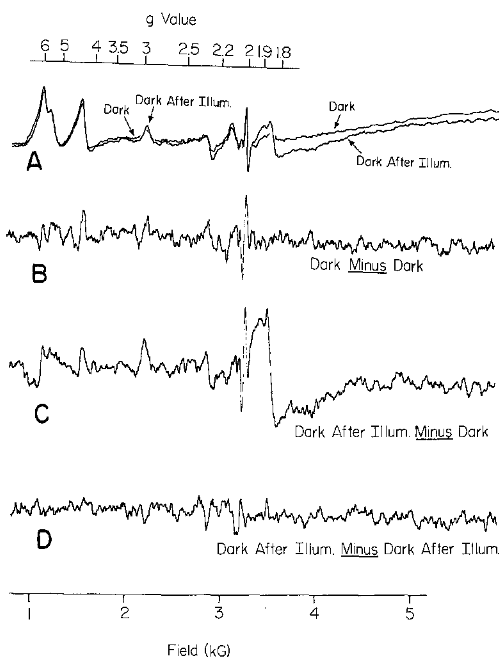

Figure 1. ESR spectra of subchromatophores (Fraction A) from Chromatium D at liquid helium temperatures. The subchromatophores (BChl 0.54 mM) were poised in oxidation-reduction potential region II at -60 mV before freezing in the dark. The following redox mediators were present: phenazine ethosulphate 30 μM, duroquinone 40 μM, pyocyanine 15 μM, 2-hydroxy-1,4-naphthaquinone 40 μM. Relevent ESR spectrometer settings were, modulation amplitude 32 G and microwave power 2.00 mV. Part A. ESR spectra of subchromatophores maintained in the dark, and the sample after a brief period (∿ 3 seconds) of illumination at liquid helium temperatures. The light was from a Unitron tungsten lamp (8v;5A) passed through a Wratten 88A filter and 2 cm of water. Parts B and D are control ESR difference spectra obtained with the CAT. Part C is the light minus dark ESR difference spectrum of the two spectra shown in A.

The midpoints potentials (pH 7.4) of cytochrome c_{553} and X measured at low temperatures are +10 mV and -135 mV respectively (3). Thus if the light induced signals are associated with both oxidized cytochrome c_{553} and reduced X, they ought to be resolvable by dark chemical oxidation-reduction techniques. This is shown in Figure 2. The signals g 2.9 and 2.25 are evident at +100 mV (region I; see ref. 2) when cytochrome c_{553} is essentially oxidized; at -60 mV (region II) when the cytochrome is approximately 90% reduced the signals are much diminished and at -280 mV (region III) they are not detectable. The g 1.82 signal is only significant in the -280 mV spectrum; at this potential X is over 99% reduced.

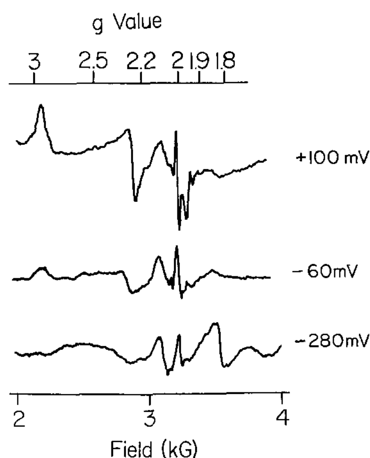

Figure 2. ESR spectra at liquid helium temperatures of subchromatophores from Chromatium D poised in oxidation-reduction potential regions I (+100 mV), II (-60 mV) and III (-280 mV). The conditions as in Figure 1 except the following additional mediators were present: phenazine methosulphate 30 μM, anthraquinone-2-sulphonate 30 μM and benzyl viologen 30 μM.

If the g 1.82 signal is associated with the reduced primary electron acceptor, then at potentials when cytochrome $\underline{c}_{553}$ is chemically oxidized before freezing (region I) the signal will be reversibly generated by light. This appears to be the case. In subchromatophores poised at +150 mV the signal is formed in light and decays in the dark at a rate within the instrumental rise-time (time constant 0.3 sec.). Under conditions where X is reduced (either photochemically in region II or chemically in region III) no further signals are generated in the light except the bacteriochlorophyll triplet (2). These facts are consistent with the g 1.82 signal being the reduced primary electron acceptor in Chromatium D. The position of the signal suggest it is that of a reduced iron-sulphur protein.

Experiments analogous to those performed on Chromatium D have been performed on chloroplasts prepared from spinach. Figure 3 shows the ESR difference spectrum "dark after illumination *minus* dark before illumination" which was obtained in the same way as described in Figure 1C. The signal is formed irreversibly at a rate which is less than the instrumental rise-time. A similar signal is generated irreversibly on illumination of chloroplasts

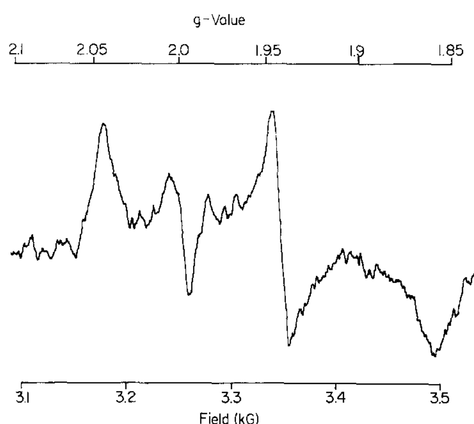

Figure 3. The light minus dark ESR difference spectrum of chloroplasts from spinach poised at +5 mV before freezing. The chlorophyll concentration was approximately 1.5 mM. The experimental approach was the same as described in Figure 1 except that the ESR spectrometer was set at a modulation amplitude 10 G and microwave power 100 mW.

which have been poised at potentials in the region +350 mV to -250 mV before freezing.

The amplitudes of the signals from both plant and bacterial preparations were sensitive to the temperature of the sample. Of the two, the *Chromatium* D signal displayed a significantly greater temperature dependence. Studies of the relationship of the signal to the microwave power revealed no appreciable saturation of either signals at microwave power settings of 50 mW.

Discussion

The primary electron acceptor of reaction center chlorophyll in various photosynthetic systems has been defined indirectly by the influence of its oxidation-reduction state on the photoreactions of the reaction center molecule (11-15) or the reactions of closely linked carotenoid (3,16) and cytochrome moieties (3,17,18). Also, a very broad light induced g 2 ESR signal at 1.4° K (19), and absorption changes at 450 nm (20), observed in

reaction center preparations from the photosynthetic bacterium Rhodopseudomonas spheroides have been attributed to the oxidation-reduction reactions of the primary electron acceptor. In spinach chloroplasts at 77° K the irreversible light generation of reduced iron-sulphur protein (observed by ESR at 25° K) has been reported (21); in this case many minutes were required to generate the signal employing a very high intensity light. Also in plant preparations, the rapid laser induced absorbance change at 430 nm has been considered as the reduction of primary electron acceptor of photosystem I (22).

The results with Chromatium D presented in this communication satisfy several criteria which when considered altogether serve to identify the primary electron accepter of reaction center Bacteriochlorophyll:

a) The g 1.82 signal is evident in a solubilized photoactive subchromatophore preparation the constitution of which has been largely characterized (6).

b) The light induced behavior of the g 1.82 signal conforms completely to the defined light induced oxidation-reduction reactions of P883 and cytochrome c_{553} at liquid helium temperatures (2-4). In region I the g 1.82 signal is generated reversibly on illumination; in region II it is generated irreversibly; in region III no further light induced g 1.82 signal is observed on illumination.

c) The g 1.82 signal can be induced in the dark by chemical reduction. This occurs over an oxidation-reduction potential range appropriate for the reduction of X, as defined by independent measurement of its midpoint potential (3).

The possibility of the signal arising from the reduction of a secondary electron transfer intermediate can be ruled out on the basis of our current knowledge of the low temperature reactions of Chromatium D (1-4,23). For instance, in region II a 20 nsecond laser pulse activates P883 oxidation and simultaneous X reduction; this is followed by the irreversible oxidation of cytochrome c_{553} and concomitant reduction of $P^{+}883$. If it happened that

X was subsequently reoxidized by a secondary electron acceptor at these low temperatures this would render X available to accept an electron from P883 when activated by a second laser pulse. Since no P883 oxidation is elicited by a second or subsequent laser pulses it is reasonable to consider that the g 1.82 signal is the reduced primary electron acceptor.

The properties of the ESR signal lead to the conclusion that the primary electron acceptor is an iron-sulphur protein. These compounds are usually low potential one-electron oxidation-reduction agents. In the reduced form they display similar ESR absorption in the g 1.8 - 2.1 region; their ESR signals are often unusually temperature sensitive (see ref. 24). The position of the signal observed in _Chromatium_ D is lower in g-value than the values reported for isolated, soluble iron-sulphur proteins. This difference may be a direct consequence of the strong binding of the protein to the reaction center. In this regard the iron-sulphur moiety of mitochondrial complex III also has a somewhat lower g-value (25).

The ESR spectrum displayed by the spinach chloroplasts is essentially the same as that reported by Malkin and Bearden (21). It is a typical green plant iron-sulphur protein. While it is a promising candidate as an early electron acceptor at the electronegative end of Photosystem I, further evidence is needed before the spectrum can be identified with the primary electron acceptor.

Acknowledgements

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