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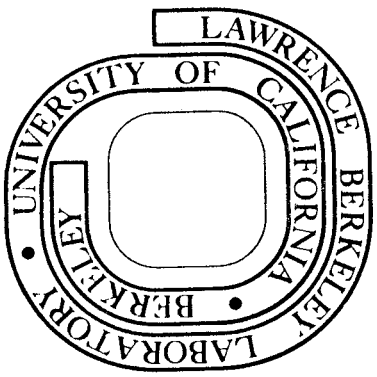

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MEMBRANE-BOUND IRON-SULFUR CENTERS IN PHOTOSYNTHETIC SYSTEMS

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I. Introduction

The isolation and characterization of the iron-sulfur protein, chloroplast ferredoxin, in 1962 [1] initiated the investigation of the role of iron-sulfur proteins in photosynthetic processes. This work culminated in the identification of soluble proteins of the iron-sulfur type in all photosynthetic organisms and elucidation of a multitude of different electron

transfer reactions in which these proteins participate (for reviews, see refs. 2 and 3). The ferredoxins, although of different chemical nature, were soluble proteins in that they could readily be isolated after separation of the soluble cell fraction from the chlorophyll-containing membrane fragments [4]. The fragments were known to contain membrane-bound electron carriers which were essential for photosynthetic electron transport reactions, but there was no indication that they might contain membrane-bound iron-sulfur groups.

The application of electron paramagnetic resonance (EPR) spectroscopy to the study of iron-sulfur proteins was first made with mitochondria and submitochondrial particles (see refs. 5–7 for recent reviews) and this was followed by characterization of soluble iron-sulfur proteins of the ferredoxin type as well as bound centers in photosynthetic systems. In the case of bacterial chromatophores and chloroplasts, the distinctive EPR properties of the iron-sulfur proteins made possible the detection of bound iron-sulfur centers in these systems, which were previously thought to contain only soluble ferredoxins. It is, however, more appropriate at the present time to denote these groups as iron-sulfur centers or clusters, rather than iron-sulfur proteins, since the association of these centers with specific proteins is not well established because extraction, purification and characterization of iron-sulfur proteins from photosynthetic membranes has only been accomplished in a few instances.

As in mitochondria, a multiplicity of iron-sulfur centers, firmly bound to membranes, has been found in chromatophores from photosynthetic bacteria as well as in chloroplasts and membrane fragments from other oxygen-evolving organisms, such as blue-green algae. In this review, we will consider the properties of these centers and their proposed roles in primary photochemical processes and secondary electron transfer reactions of photosynthetic organisms.

II. Membrane-bound iron-sulfur centers in oxygen-evolving organisms

IIA. Low-potential iron-sulfur centers

IIA-1. A bound iron-sulfur center as a stable primary electron acceptor of chloroplast Photosystem I. The identification of bound iron-sulfur centers in chloroplasts was first made in 1971 on the basis of low-temperature EPR analysis [8]. As shown in Fig. 1, illumination of intact chloroplasts at 10 K produces a paramagnetic species with EPR g values ($g_x = 1.86, g_y = 1.94, g_z = 2.05$) characteristic of the one-electron reduction of an iron-sulfur center (see ref. 5 for a detailed description of the EPR properties of iron-sulfur centers). Although the initial report of the detection of this center in chloroplasts utilized illumination at 77 K, subsequent technical improvements have allowed for illumination of samples directly in the EPR cavity at lower temperatures, such as that shown in Fig. 1.

The g-values of the photoreduced iron-sulfur center are similar to, but not identical with, those of soluble chloroplast ferredoxin ($g_x = 1.89, g_y = 1.96, g_z = 2.05$), the only known iron-sulfur protein in chloroplasts at the time these experiments were done. The linewidths of the two sets of signals are significantly different: the soluble ferredoxin signal has a linewidth of approximately 50 G while that of the above component is only 15 G. It was therefore suspected, and subsequently proven, that this new center observed in intact chloroplasts did not originate from the soluble ferredoxin. Since the latter protein can be completely removed from chloroplasts by washing the membrane fragments with dilute buffer, it was possible to show that the EPR intensity of the new component was unaltered by such treatment. In addition, as will be discussed in more detail below,

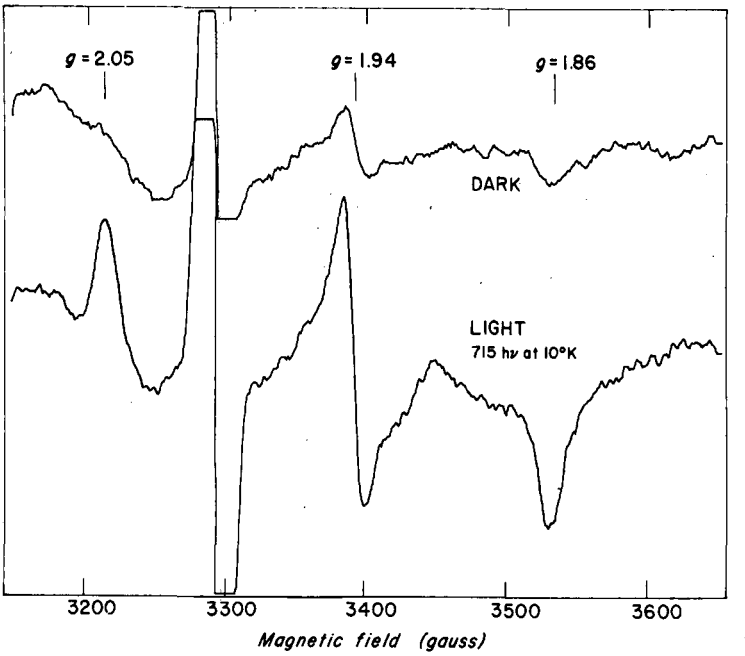

Fig. 1. Photoreduction of a bound iron-sulfur center in intact chloroplasts after illumination at 10 K with 715-nm light.

the new iron-sulfur center was found in preparations of subchloroplast fragments which were totally devoid of soluble ferredoxin. Thus, the new center was first referred to as a 'bound ferredoxin' to distinguish it from the 'soluble' ferredoxin [8], but because the chemical nature of this center has not been characterized in the isolated state, we prefer to denote the component in more general terms as a 'bound iron-sulfur center' and thereby hopefully avoid any confusion which might arise about the application of the term 'ferredoxin'.

As well as detecting the presence of a bound iron-sulfur center in chloroplasts, the early work also revealed an important property of this center: the center could be photoreduced at cryogenic temperatures. It is widely accepted, although not conclusively proven, that components in photosynthetic systems which undergo photoreactions at cryogenic temperatures are closely associated with primary photochemical events. The assumption is that normal diffusion-limited chemical reactions do not occur at cryogenic temperatures while reactions linked to photon absorption can still occur at an undiminished rate. Although recent work has tended to show this to be an over-simplification, the photoreduction of the iron-sulfur center at cryogenic temperatures would tend to relate the process to a primary photochemical event in chloroplasts.

a. Association with the Photosystem I reaction center. Further investigation has documented the association of the photoreducible iron-sulfur center with the Photosystem I reaction center. Initial evidence [9] was obtained in studies in which chloroplast samples were illuminated with monochromatic light. As shown in Fig. 1, far-red light (715 nm), which activates primarily Photosystem I, effectively reduces the bound iron-sulfur center. Red light, which activates both photosystems, produces no further photoreduction after far-red illumination. This finding directly relates the photoreducible iron-sulfur center

with the Photosysem I reaction center and does not support any models in which a bound iron-sulfur center is reduced uniquely by Photosystem II.

Studies involving subchloroplast fragments enriched in either the Photosystem I or Photosystem II reaction center have confirmed the association of the bound iron-sulfur center with Photosystem I [9,10]. Various types of Photosystem I subchloroplast fragments (prepared with detergents or by the French pressure cell method) have all shown the presence of the bound iron-sulfur center and it has been possible to demonstrate its photoreduction in these preparations [9–13]. As but one example, Fig. 2 shows that in a Photosystem I preparation prepared with lauryl dimethylamine oxide [11], which contains 1 *P*-700 per 35 chlorophyll molecules, the bound iron-sulfur center can be photoreduced at 20 K after a single flash of 730-nm light from a dye laser. The *g*-values of the center in the subchloroplast preparation are the same as those in unfractionated chloroplasts (compare with Fig. 1), but some line broadening has been observed in these enriched preparations (see ref. 13). In contrast to these results, one detergent, sodium dodecyl sulfate (SDS), has unusual effects in terms of the Photosystem I reaction center complex, and these will be discussed in greater detail in a subsequent section.

Bound iron-sulfur centers have also been observed in a number of different preparations from algal species, including green algae [14], and blue-green algae [14,15], as well as in enriched Photosystem I preparations from such organisms [13]. In contrast to this wide-spread distribution, no bound iron-sulfur centers are observed in a highly purified Photosystem II reaction center complex prepared from spinach chloroplasts with Triton X-100 [16].

Although quantitative EPR analyses of various photosystem I enriched preparations have not been carried out in a systematic manner, it appears on a qualitative basis that as

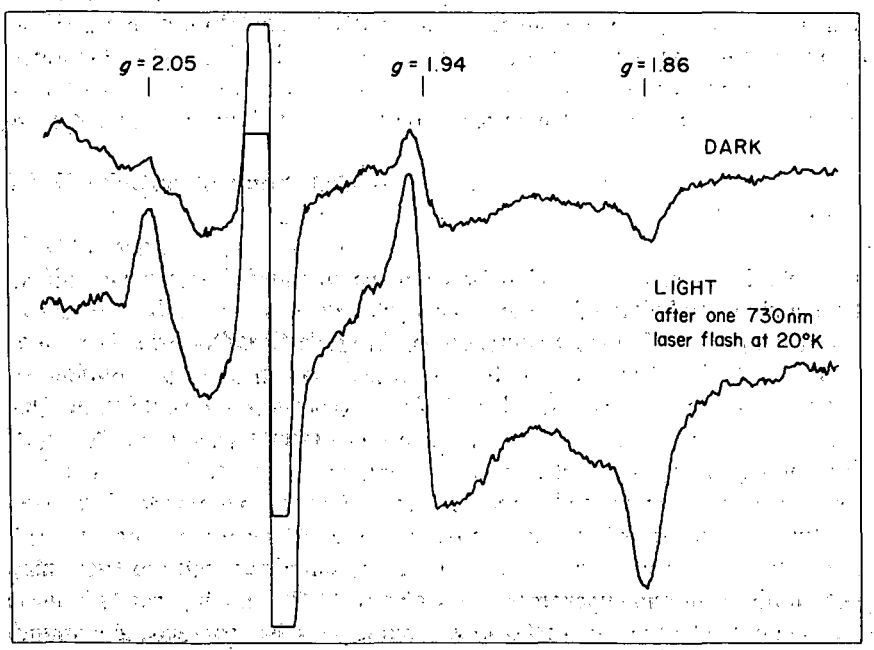

Fig. 2. Photoreduction of a bound iron-sulfur center in a Photosystem I subchloroplast fragment prepared with lauryldimethylamine oxide after flash activation with 730-nm light at 20 K. From ref. 11.

TABLE I

CHLOROPHYLL, *P*-700, NON-HEME IRON AND ACID-LABILE SULFIDE CONTENT OF CHLOROPLASTS AND A PURIFIED PHOTOSYSTEM I FRAGMENT

From Ref. 19.

Fraction	Chlorophyll/*P*-700	Non-heme iron/*P*-700	Acid-labile sulfide/*P*-700
Thylakoid membranes	345.0	17.0	14.2
DEAE-Biogel fraction A-II	27.1	10.6	10.3
DEAE-Biogel fraction A-III	22.6	10.7	10.5

the reaction center becomes more enriched in such preparations (based on *P*-700 content), the enrichment of the bound iron-sulfur center shows a parallel increase. In the case of the center shown in Fig. 2, the bound iron-sulfur center is approx. 10-fold enriched on a chlorophyll basis when compared with unfractionated chloroplasts and this is similar to the enrichment of *P*-700 in this particular preparation.

It is also possible to analyze preparations for their content of non-heme iron and acid-labile sulfide in order to quantitate the bound iron-sulfur center concn. [8,17–19]. This type of analysis has recently been reported in detail by Golbeck et al. [19] for thylakoid membranes and an enriched Photosystem I fragment isolated after treatment with Triton X-100. As shown in Table I, the most purified fractions which are obtained after chromatography on DEAE-Biogel contain approximately one *P*-700 per 25 chlorophyll molecules. Such fractions have a non-heme iron and acid-labile sulfide content of 10 mol per mol of *P*-700. It can be calculated from the concentration of these components in unfractionated thylakoid membranes that this represents a 10-fold enrichment; this is comparable to the reported enrichment of *P*-700 in the preparation.

b. Correlations with *P*-700. Because of the association of the bound iron-sulfur center with Photosystem I, numerous types of correlations between the iron-sulfur center and *P*-700, the reaction center chlorophyll of Photosystem I, have been described. The general object of this work has been to test the role of the photoreducible bound iron-sulfur center in the primary photochemistry of Photosystem I as a reaction center partner for *P*-700. Many of these early studies, which will be described in detail, supported the assignment of the iron-sulfur center as this reaction partner and indicated this center functioned as the Photosystem I primary electron acceptor. More recent work, however, has led to a different conclusion and has suggested the possible existence of an intermediate which may be photoreduced prior to the iron-sulfur center. Thus, the question of the 'true' primary electron acceptor has become a matter of controversy centering, in some respects, on difinitions for the prerequisites of such a species. Before turning to this problem, it would be well to review some of the findings which have led to this current state.

An important finding which was consistent with a role for the iron-sulfur center in the Photosystem I primary photochemistry was the observed stoichiometry between photoreduced iron-sulfur center and photooxidized *P*-700 [20]. As shown in Table II, quantitative EPR estimation of the number of photoreduced iron-sulfur centers and photooxidized *P*-700 molecules in chloroplasts and digitonin Photosystem I fragments showed a 1:1 correspondence after illumination with 715-nm light at 25 K.

It is now well documented that under the conditions of the quantitative EPR study there is no reversibility of the charge separation, that is, photooxidized *P*-700 and photo-

TABLE II

STOICHIOMETRY OF PHOTOOXIDIZED *P*-700 AND PHOTOREDUCED IRON-SULFUR CENTER AFTER ILLUMINATION WITH 715-nm LIGHT AT 25 K

From Ref. 20.

Preparation	Chlorophyll (mM)	*P*-700 (μM)	Chlorophyll / *P*-700	Chlorophyll / Fe-S center	Reduced Fe-S center / Oxidized *P*-700
Intact chloroplasts					
1	4.0	6.8	590	430	1.37
2	5.0	13.1	380	300	1.25
3	3.7	9.8	380	340	1.11
4	2.5	5.4	460	380	1.22
5	4.1	12.1	340	280	1.20
Average			430 ± 75	350 ± 50	1.23
D-144 Photosystem I fragments					
1	3.0	15.8	190	170	1.11
2	3.3	20.6	160	210	0.78
3	3.7	26.4	140	150	0.94
Average			160 ± 15	175 ± 20	0.91

reduced iron-sulfur center are stable in the dark after the cessation of illumination. This reaction becomes reversible as the temperature is raised to near liquid nitrogen temperature and at temperatures from 77 to 150 K, a back reaction arising from a charge recombination between these two species has been demonstrated [21–23]. As shown in Fig. 3, there is an approximately 40% decay of $P\text{-}700^+$ in the dark at 150 K (after illumination at 20 K to produce the charge separation), and this can be correlated with an approximate 40% decay in the reduced iron-sulfur center under the same conditions (Fig. 4). The decay kinetics of $P\text{-}700^+$ at 150 K are clearly biphasic, as shown in the kinetic study of Fig. 5. The nature of the biphasic decay has not been adequately explained other than that a 'heterogeneity' exists in the Photosystem I reaction center complex [24], presumably arising as a consequence of the freezing of the samples.

Conflicting results have been presented on the light-on kinetics for the formation of

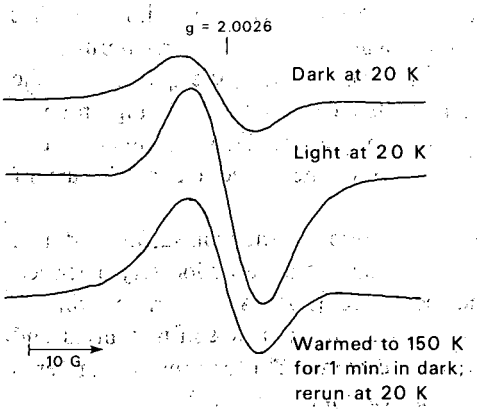

Fig. 3. Reversibility of the photooxidation of *P*-700 in the dark in a Photosystem I subchloroplast fragment at 150 K.

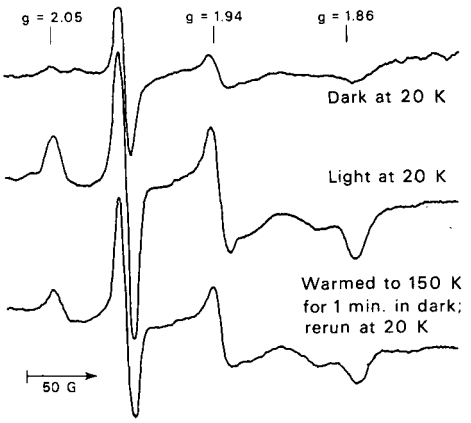

Fig. 4. Reversibility of the photoreduction of a bound iron-sulfur center in the dark in a Photosystem I subchloroplast fragment at 150 K.

P-700$^+$ and reduced bound iron-sulfur center. Visser et al. [22] have shown that at low light intensities, the kinetics of the appearance of the two species are identical; McIntosh et al. [25] have studied the flash-induced appearance of both signals and concluded that the relative quantum efficiencies differ by a factor of 5. Since these two experiments were not performed in an identical manner, it is difficult to compare the results. It would appear that a reinvestigation of this problem under conditions where the light intensity is varied over a wider range of intensities would be desirable before attempting to assign or dismiss components as primary reactants on the basis of such measurements.

Additional evidence confirming the close association of the photoreducible bound iron-sulfur center with *P*-700 has come from recent work in which the iron-sulfur center has been specifically modified. It has been possible to use the detergent, SDS, to alter the iron-sulfur center in the Photosystem I reaction center complex from chloroplasts [26] and from the blue-green alga, *Phormidium luridium* [13]. In the latter case, a Triton X-100 Photosystem I complex (containing one *P*-700 per 40 chlorophyll molecules when assayed photochemically at 300 K) could be prepared.

The Triton preparation displays photooxidation of *P*-700 and photoreduction of the

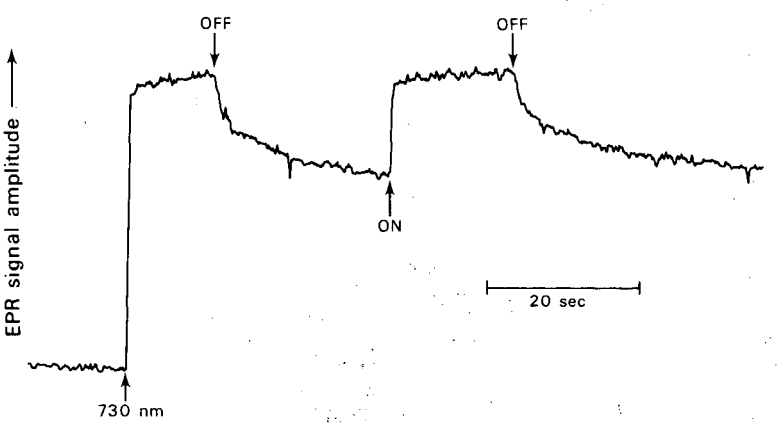

Fig. 5. Kinetics of *P*-700 formation and decay in Photosystem I subchloroplast fragments at 150 K.

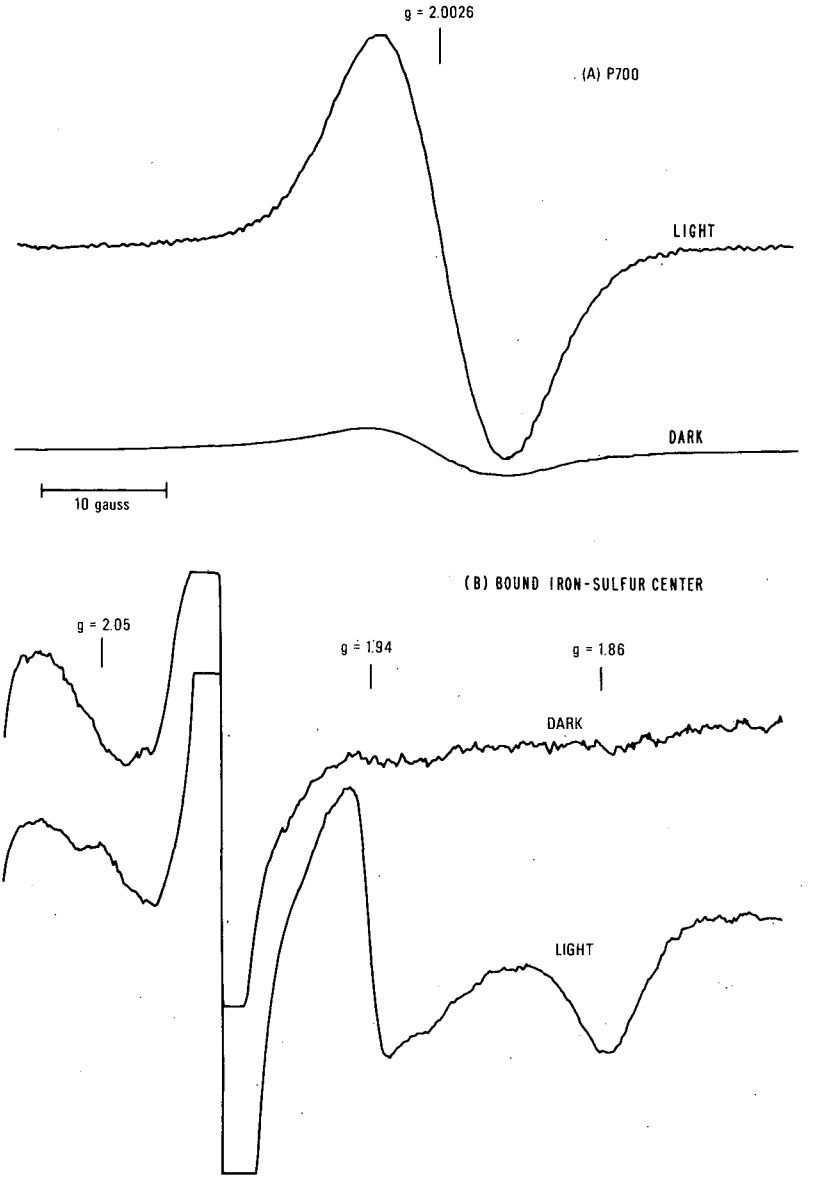

Fig. 6. Photochemical reactions at 15 K in a Triton *P*-700-chlorophyll *a*-protein complex from *Phormidium luridium*. From ref. 13.

bound iron-sulfur center at 15 K, as shown in Fig. 6. The second complex, prepared from the same organism but with SDS, shows photochemical activity at 300 K in that *P*-700 photooxidation still occurs, but when examined for low-temperature photoactivity (Fig. 7), the preparation is inactive in that it shows no *P*-700 photooxidation or iron-sulfur center photoreduction. Comparison of the two preparations after chemical reduction (with dithionite plus methyl viologen) shows that the SDS preparation lacks chemically detectable iron-sulfur centers while these centers are present in the Triton X-100 preparation.

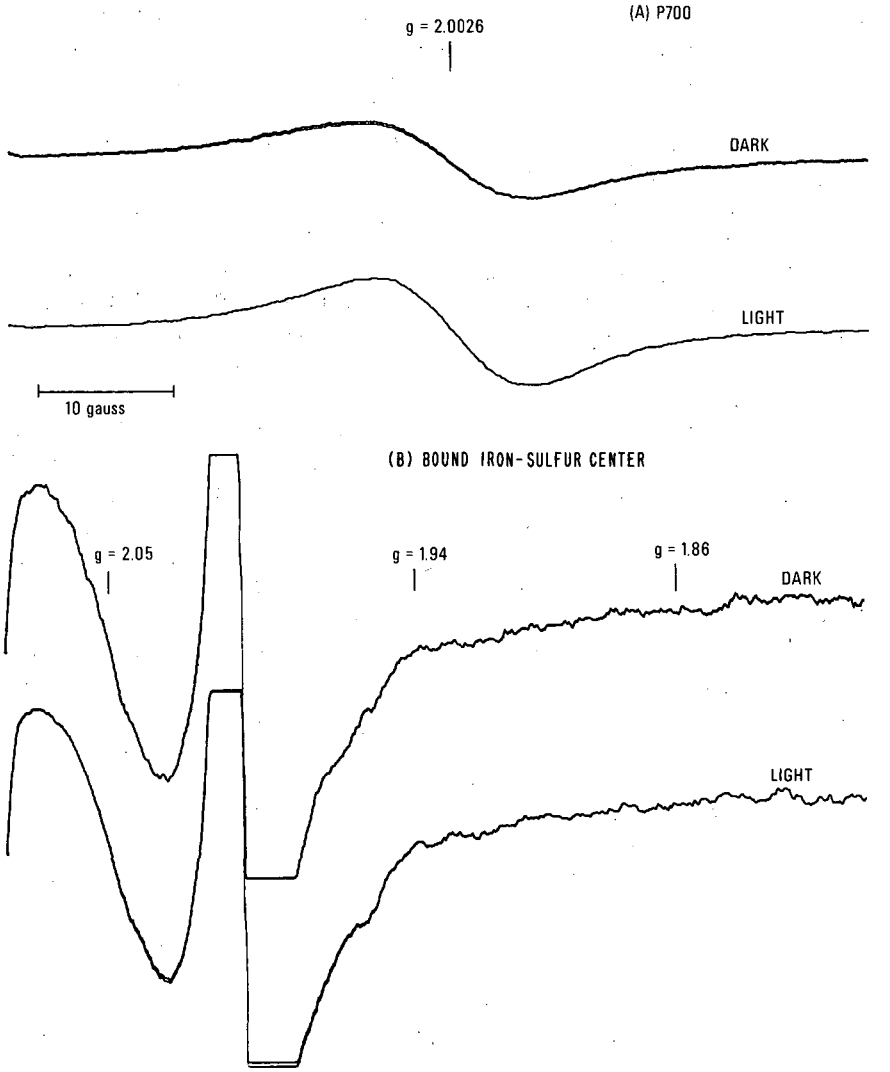

Fig. 7. Photochemical reactions at 15 K in a sodium dodecyl sulfate-*P*-700-chlorophyll *a*-protein complex from *Phormidium luridium*. From ref. 13.

Similar results have been obtained with a digitonin Photosystem I preparation which is subsequently treated with SDS [26]. These results indicate that in order to obtain a stable charge separation at cryogenic temperatures, the presence of a bound iron-sulfur center is required. Another important point which has emerged from this work is that *P*-700 photochemical activity at physiological temperatures does not necessarily correlate with photochemical activity at cryogenic temperatures. Although both blue-green algal Photosystem I preparations, as well as the SDS-treated chloroplast preparation, show room temperature photooxidation of *P*-700, presumably due to the transfer of an electron to some diffusable electron acceptor which can function at 300 K but not 15 K, only the 'native' electron acceptor can function at cryogenic temperature and the absence

of this acceptor prevents a stable charge separation. This conclusion has important consequences for future investigations of the Photosystem I reaction center since most workers routinely monitor *P*-700 photoactivity at 300 K as a measure of reaction center concentration; this assay may, however, not give a reliable estimate of the 'native' reaction centers which contain components required for a stable charge separation. It is also possible that a transient charge separation will occur in these altered preparations followed by a rapid back-reaction due to charge recombination, and a recent report by Mathis et al. [26a] has shown that in a *P*-700-chlorophyll protein complex isolated with SDS from tobacco chloroplasts, a rapid and reversible photooxidation of *P*-700 does occur. A back-reaction with a half-time of 10 μs at 294 K and 550 μs at 5 K could be observed after laser flash activation, indicating a transient charge separation does occur in the absence of bound iron-sulfur centers and that an alternate electron acceptor must be present in such preparations.

Another procedure which specifically modifies the iron-sulfur centers of Photosystem I is treatment with high concentrations of urea in the presence of ferricyanide [19,27]. This procedure apparently converts the acid-labile sulfide of the iron-sulfur centers to the S^0 state and results in loss of the ability of *P*-700 to be photooxidized at 300 K. A good correlation has been found for the decrease in *P*-700 photoactivity and the decrease in the content of acid-labile sulfide of a Triton Photosystem I preparation (1 *P*-700 per 25 chlorophyll molecules) during treatment with urea/ferricyanide. *P*-700 does not appear to be altered by this treatment since detection from oxidized minus reduced chemical difference spectra revealed no decrease in the amount of this component. One significant advantage of the urea/ferricyanide treatment as compared with the SDS treatment is that the former is reversed by treatment of the fragments with dithiothreitol, i.e. acid-labile sulfide is restored and *P*-700 photochemical activity is regained (see ref. 19). Although detailed physical studies utilizing EPR spectroscopy have not yet been done on the urea/ferricyanide-treated material to determine the nature of the modification, this system seems particularly attractive for future investigations of the role of iron-sulfur centers in Photosystem I.

c. On the role of the photoreducible iron-sulfur center in Photosystem I. The early evidence on the irreversibility of the Photosystem I charge separation at temperatures in the liquid helium range led to the proposal that the photoreducible iron-sulfur center functions as the primary electron acceptor of this photosystem [8–10]. The studies using SDS-modified Photosystem I preparations from chloroplasts and blue-green algae as well as those using urea/ferricyanide to modify the iron-sulfur centers, demonstrate that in order to obtain a stable charge separation in the Photosystem I light reaction, the presence of a bound iron-sulfur center is a prerequisite. What has emerged from this body of work is the concept that the electron lost from *P*-700 during the Photosystem I primary charge separation reaches the bound iron-sulfur center but that a barrier exists for the subsequent transfer of this electron. This is particularly evident from the studies of the charge separation at liquid helium temperature where photooxidized *P*-700 and photoreduced iron-sulfur center accumulate after illumination.

The term 'primary electron acceptor' implies that the reduced species is the first reduced product formed during the charge separation. Evidence to be discussed in greater detail in a subsequent section indicates that an intermediate may be photoreduced prior to the iron-sulfur center. An analogous situation has been found in the reaction center from photosynthetic bacteria in which the component known as 'the primary electron acceptor' (probably an iron-ubiquinone complex) is not the first reduced species

formed but an intermediate involving bacteriopheophytin is reduced prior to the iron-ubiquinone complex [28]. It is clear from the studies which have been discussed that a bound iron-sulfur center functions as a 'stable' electron acceptor in the Photosystem I reaction center complex in a way apparently analogous to the iron-ubiquinone complex of the bacterial reaction center. This assignment does not preclude the identification of intermediates which might be formed prior to the reduced iron-sulfur center although it does suggest that an obligate, rapid electron transfer from such an intermediate to the iron-sulfur center would occur, even at cryogenic temperatures. Possible models involving such components will be discussed in Section II.A-5.

IIA-2. A second low-potential iron-sulfur center in the Photosystem I reaction center complex. a. Identification based on EPR spectroscopy. Concomitant with the identification of the photoreducible iron-sulfur center, a second iron-sulfur center was also detected using EPR spectroscopy [8,10]. As shown in Fig. 8, reduction of Photosystem I fragments in the dark with a strong reductant (hydrogen gas plus hydrogenase or sodium dithionite and methyl viologen) produces an EPR spectrum similar to that observed after photoreduction but showing additional resonance lines at $g = 1.92$ and 1.89. Longer incubation with reductant produces a 'fully reduced' spectrum which has g-values at 2.05, 1.94, 1.92 and 1.89. The latter spectrum is unusual in that the g-value at 1.86 has disappeared, a matter for which there is no clear explanation.

It was noted in an early report [8] that the fully reduced spectrum characterized by 4 g-values cannot arise from a single, isolated iron-sulfur center since a maximum of 3 EPR g-values are obtained from an $S = \frac{1}{2}$ transition metal ion, and it was proposed that such a spectrum arises from the overlap of 2 different spectra, originating from 2 differ-

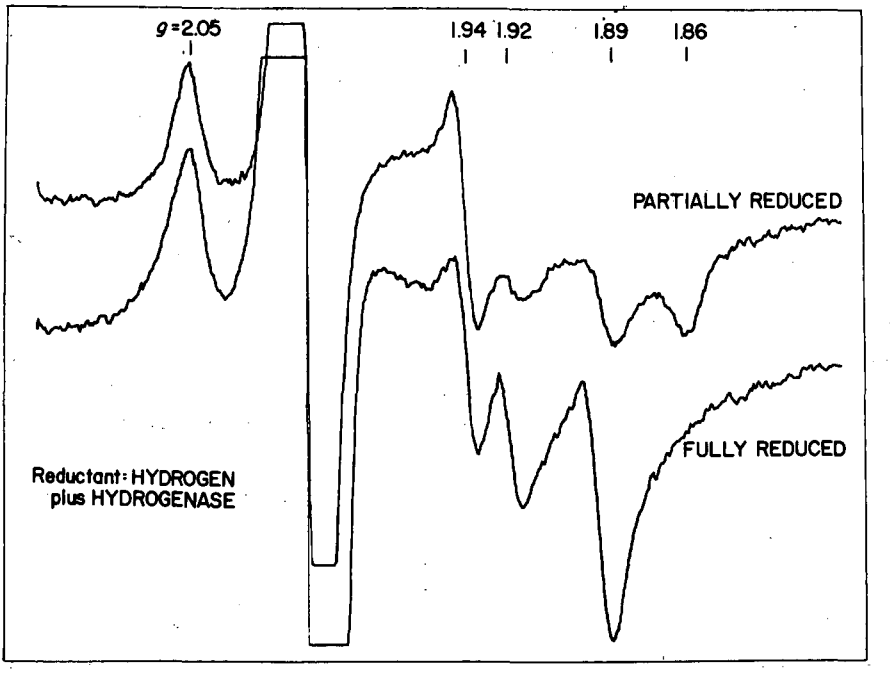

Fig. 8. EPR spectra of the iron-sulfur centers of chloroplasts after reduction in the dark with hydrogen and hydrogenase in the presence of methyl viologen.

ent iron-sulfur centers [8]. Subsequent work by other groups [29,30] has tended to confirm this interpretation of the chemically-reduced spectra. The 2 iron-sulfur centers are commonly referred to as A (g-values of 2.05, 1.94 and 1.86) and B (g-values of 2.05, 1.92 and 1.89) with Center A being the photoreducible component discussed in the preceding section.

b. Oxidation-reduction properties of Centers A and B. The presence of two different iron-sulfur centers in Photosystem I has been confirmed by determination of the midpoint oxidation-reduction potentials (E_m) of the centers [29,30]. As can be noted from Fig. 7, Center A appears to be reduced chemically prior to Center B, suggesting a more positive E_m. Ke et al. [29] and Evans et al. [30] have determined the E_m values of these centers and, although the two sets of values are not identical, they are similar, as shown in Table III. Although Ke and co-workers reported n-values of 2 for both centers, Evans and co-workers obtained results which were compatible with $n = 1$ titration curves for both centers, and the latter values seem more reasonable in terms of the known oxidation-reduction reactions of other iron-sulfur centers.

One interesting feature which has emerged from these studies concerns the disappearance of the $g = 1.86$ signal over the course of the reductive titration. As noted in Fig. 8, this g-value is apparent in the spectrum of a partially reduced sample but is absent from the spectrum of a fully reduced sample. On the basis of qualitative examination of the intensity of the peaks in the fully reduced spectrum, it appears the $g = 1.86$ signal undergoes a g-value shift to 1.89 although the other g-values of Center A remain unchanged. This conclusion has not been tested by careful simulation of the EPR spectra but it has been shown that a 1:1 relationship exists in the stoichiometry of Center A and Center B [31] and the disappearance of the g-value at 1.86 correlates well with the appearance of the $g = 1.89$ signal of reduced Center B [29,30]. The possible significance of this g-value shift will be discussed in greater detail in a subsequent section.

c. Photoreducibility of Center B. Conflicting claims have been made as to the extent of the photoreducibility of Center B at cryogenic temperature. Ke et al. [29] first showed that little or no Center B photoreduction occurs in Photosystem I fragments poised at oxidation-reduction potentials where Center A is predominantly reduced. In contrast, Evans and Cammack [32] have shown some photoreduction of Center B in Photosystem I fragments poised at −560 mV prior to freezing and low temperature illumination; at this potential Center A would be approx. 75% reduced. However, the extent of the reduction of Center B seems small and does not approach the stoichiometric amount of Center A photoreduced under mildly reducing conditions.

We have observed a small but variable amount of photoreduction of Center B at cryogenic temperatures in various Photosystem I subchloroplast fragments but this amount is

TABLE III

PROPERTIES OF THE LOW-POTENTIAL IRON-SULFUR CENTERS OF THE PHOTOSYSTEM I REACTION CENTER COMPLEX

Center	EPR g-values	E_m (mV)	ref.
A	2.05, 1.94, 1.86	−530	29
		−553	30
B	2.05, 1.92, 1.89	−580	29
		−594	30

approx. 10–25% of the amount of Center A photoreduced. A recent set of experiments with whole cells of the green alga *Dunaliella parva* show a nearly equal photoreduction at 10 K of Center A and B (Malkin, R. and Bearden, A.J., unpublished). These results represent the largest amount of photoreducible Center B which we have observed and may argue for a role of this center in the primary charge separation. However, because of the variability of the results concerning the reactions of Center B at cryogenic temperatures, it is probably premature to assign it a role in the Photosystem I primary reaction (see Section IIA-5).

The photoreduction of Center B as well as Center A can be demonstrated in chloroplast fragments at physiological temperatures. In the presence of an electron donor system and by freezing samples during illumination, it is possible to trap the carriers under steady-state conditions. Spectra similar to those obtained after dark chemical reduction were obtained using electron donors such as reduced dichlorophenolindophenol with Photosystem I fragments, indicating that Center B can undergo photoreduction at 300 K (Malkin, R. and Bearden, A.J., unpublished).

A recent report has described the photoreduction of Center A and B in oxygen-evolving fragments from a blue-green alga [15]. Attempts were made to study shifts in steady-state levels of these centers after the addition of electron acceptor systems, but the conclusions drawn from this work are not correct because of errors in interpreting the *g*-values in the reduced EPR spectra. It is clear from Fig. 8 that the $g = 1.89$ resonance line does not originate solely from Center B but has a contribution from Center A (the shifted $g = 1.86$ resonance line) on complete reduction. Arnon et al. [15] have concluded from their studies that only Center B is involved in the photoreduction of $NADP^+$ with reduced dichlorophenolindophenol as the electron donor. This conclusion is based on an observed shift in the steady-state level of the $g = 1.89$ resonance line on the addition of an electron acceptor. Since this *g*-value does not solely originate from Center B, this conclusion is not correct. It appears to the present authors that both Center A and B undergo photoreduction with either water or reduced dichlorophenolindophenol as electron donors, based on the *g*-values at 1.94 and 1.92; both centers also undergo shifts in their steady-state levels upon the addition of electron acceptors. Thus the necessity of proposing a unique role for Center B in a noncyclic electron transport pathway is not supported by these experiments (see Section IIA-5).

d. Nature of the iron-sulfur centers. The presence of the two different iron-sulfur centers in the Photosystem I reaction center raises questions as to their chemical nature. Iron-sulfur centers in isolated proteins are known to contain either two or four iron atoms (see ref. 5), and it is presumed that the bound centers contain either 2 or 4 iron atoms. It has not yet been possible to isolate these centers in soluble proteins which retain their native properties (see Section IIA-4) so that this approach has not yielded an answer to this question.

A different approach employed by Cammack and Evans [33] utilizes reduction of the iron-sulfur centers in Photosystem I fragments after treatment with 80% dimethylsulfoxide and comparison of the EPR spectra of the treated material with those of similarly treated 'control' proteins (soluble chloroplast ferredoxin, which contains 2 iron atoms, and soluble clostridial ferredoxin, which contain 2 4-Fe centers). The conclusion from these studies was that the fragments contained 2 4-Fe centers and no 2-iron centers were present. In light of the recent evidence that 80% dimethylsulfoxide treatment can facilitate dimer-to-tetramer conversion in synthetic analogs of iron-sulfur proteins and in extrusion procedures applied to the iron-sulfur proteins [34], this conclusion may not be

fully justified. Analysis of highly enriched Photosystem I fragments shows approx. 10 atoms of non-heme iron per *P*-700 [19], a result which indicates the amount of iron is in excess of that required for two four iron centers per reaction center. Further analysis of this type will require more purified reaction center preparations.

The relationship of the 2 iron-sulfur centers (Center A and B) has also been a question of some interest. The close association of the centers is indicated by the *g*-value shift whch occurs during reductive titration, but the meaning of this effect is not yet understood. Evans et al. [30] proposed that the disappearance of the $g = 1.86$ signal of Center A during reduction of Center B is due to changes in the shape of the Center A spectrum as a result of spin-spin interactions between the two reduced centers. Such a conclusion would necessitate that the two centers are extremely close, probably in the same protein molecule. It would be suprising that such a strong interaction only results in the shift of one *g*-value and not in more pronounced spectral perturbations. In addition, Ke et al. [12] have reported that is is possible by Triton X-100 treatment of chloroplasts to obtain a cytochrome complex which contains Center B but no Center A. The reduced EPR spectrum of this preparation shows a resonance line at $g = 1.94$ as well as at $g = 1.92$ and 1.89 so that the identity of the center in the preparation with either Center A or B is not clear. It has not yet been possible to obtain subchloroplast fragments which contain only one of the two centers, and this result, although of a negative nature, again confirms the close association of the two centers and suggests their possible presence in a single protein. Clearly, these questions await future clarification.

IIA-3. An electron acceptor functioning prior to the bound iron-sulfur centers in Photosystem I. a. Background of the problem. Recent evidence has suggested a ubiquinone-iron complex to be the primary electron acceptor in the reaction center of photosynthetic bacteria [28,35–37]. Electron transfer to this acceptor is independent of temperature and once formed, the primary reactants are relatively stable. The development of picosecond laser spectroscopic techniques has allowed for investigation of the bacterial primary reaction in a time domain unattainable until only recently. These studies [38–40] have demonstrated that a short-lived intermediate, possibly involving a reduced bacteriopheophytin molecule [41], is formed in less than 10 ps and donates an electron in approximately 200 ps to the ubiquinone-iron complex. The latter carrier has usually been designated as the primary electron acceptor even though these experiments demonstrate that it is not the first reduced species formed during the charge separation. This older designation is based on the relatively greater stability of the reduced form of the ubiquinone-iron complex compared with that of the reduced form of the transient: the latter has a lifetime of nanoseconds (based on measurements of a back-reaction with $P\text{-}870^+$ after extraction of quinone or under conditions where the quinone is reduced prior to laser activation) while the half-time for the back-reaction between $P\text{-}870^+$ and the reduced ubiquinone-iron complex is approximately 30 ms [28,42]. The transient appears to function by transferring an electron to an acceptor which stabilizes the charge separation for a time long enough for chemical processes to be carried out. The existence of an intermediate in the charge separation event of photosynthetic bacteria has led to speculation about the existence of similar intermediates in the photochemical events of chloroplast photosynthesis. This will certainly be an area of active investigation in the future and the possible existence of such an intermediate in the case of the Photosystem I reaction center will be considered in greater detail because of its relevance to the function of the bound iron-sulfur centers in this charge separation.

b. Kinetic disparities between *P*-700 and bound iron-sulfur centers. The evidence

which has been described on iron-sulfur center A is consistent with a function as a 'stable' primary electron acceptor. Such an assignment in no way denies the existence of one or more intermediates which might function prior to the iron-sulfur center. Recent evidence from the laboratories of Evans [32,43–46] and Bolton [25,43,47–49] has suggested the existence of such an intermediate. The basic observation leading to this conclusion concerns the reversibility of the Photosystem I charge separation at liquid helium temperatures. A back-reaction between $P\text{-}700^+$ and reduced Center A is known to occur at higher temperatures [21–23] and results indicate a reversible reaction between $P\text{-}700^+$ and a new component 'X' may occur at temperatures as low as 10 K. As shown in Fig. 9, $P\text{-}700^+$ formation is essentially irreversible when Photosystem I fragments are illuminated after preincubation with ascorbate or some similar mild reductant; under these conditions

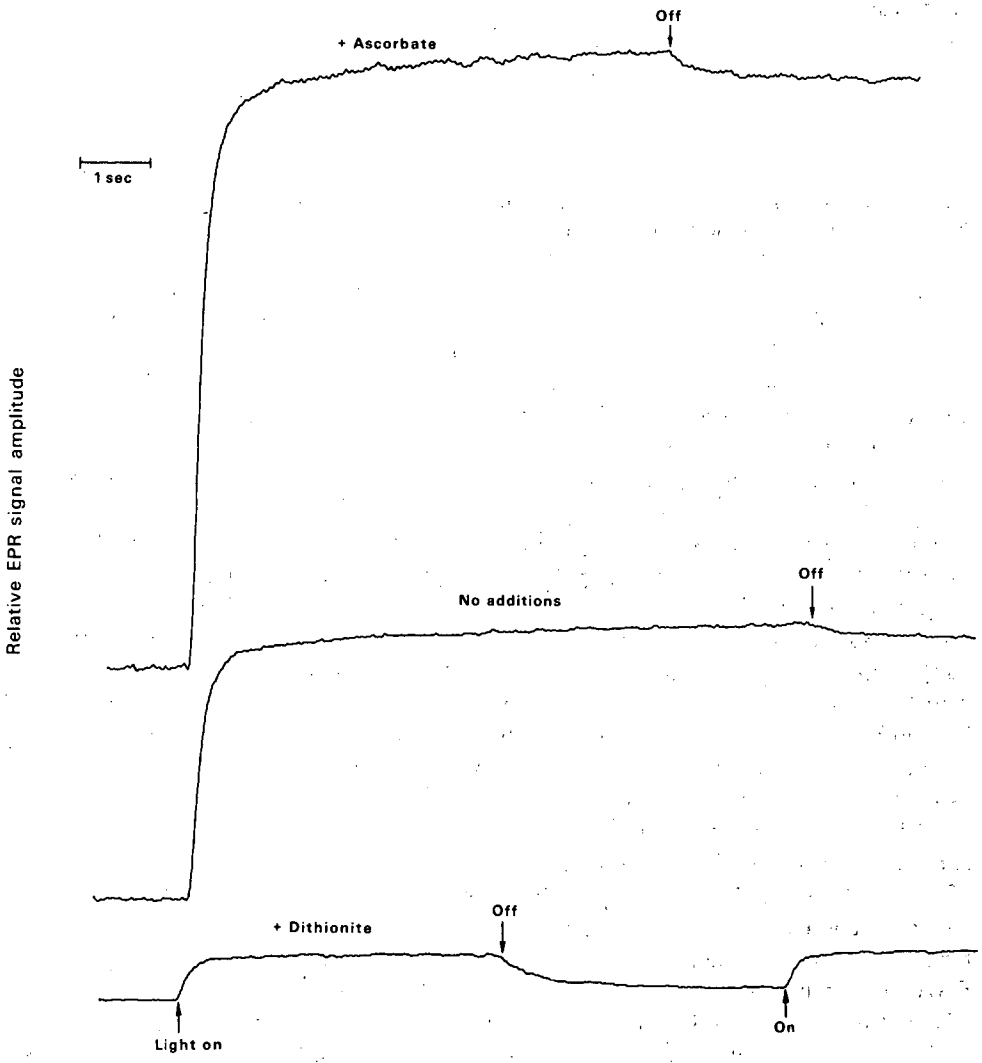

Fig. 9. Kinetics of the formation of oxidized $P\text{-}700$ at 18 K in a Photosystem I subchloroplast fragment made with lauryldimethylamine oxide in the presence of different reductants. From ref. 50.

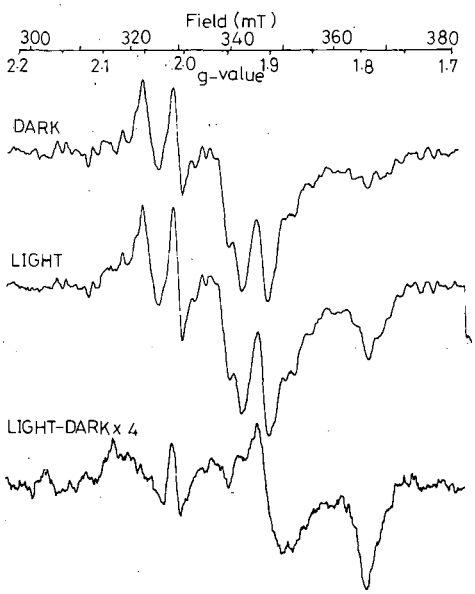

Fig. 10. The light-induced EPR spectrum of Component 'X' in *Chlorogloea fritschii.* The light minus dark spectrum of 'X' is shown in the lower trace of the figure. From ref. 44.

iron-sulfur Center A is irreversibly photoreduced. After the addition of the strong reductant, dithionite, most of the *P*-700 photooxidation is eliminated but a residual reaction does occur and this reaction is predominantly reversible. Under these conditions it has not been possible to correlate the *P*-700 changes with any changes in the oxidation-reduction state of the iron-sulfur centers. This result has led to a search for a component which is kinetically compatible with *P*-700 under conditions where the bound iron-sulfur centers are previously reduced [43–47,48].

The component 'X' discovered by Evans and Bolton has an EPR spectrum shown in Fig. 10. This component has EPR *g*-values of $g_x = 1.78$, $g_y = 1.88$ and $g_z = 2.08$. Evans [46] has suggested that this type of spectrum is similar to those of reduced iron-sulfur centers. Bolton [49], on the other hand, suggested the spectrum may originate from a quinone-iron complex similar to that found in the photosynthetic bacteria. This component has been detected by illumination at cryogenic temperatures of samples in which the iron-sulfur centers are chemically reduced by dithionite; it was also possible to trap 'X' in the reduced state by illumination during freezing under extreme reducing conditions [43–45]. In these initial studies 'X' was only observed in samples treated with dithionite, but Dismukes and Sauer [46a] have found that 'X' can be photoreduced in untreated chloroplasts or chloroplasts incubated with the mild reductant ascorbate. Kinetic studies of McIntosh et al. [47] have shown that charge recombination between X^- and $P\text{-}700^+$ occurs in approximately 500 ms in the temperature range from 5 to 10 K. This recombination rate is extremely slow in comparison with that observed for the transient and $P\text{-}870^+$ in the bacterial reaction center (in the nanosecond time range, see refs. 28 and 42), and further detailed kinetic studies are necessary in order to evaluate the significance of this reaction in relation to secondary electron transfer processes.

A general problem which has existed in the study of this new component concerns the extent of the reversible *P*-700 reaction under conditions where the bound iron-sulfur cen-

ters are not available for photoreduction. Initial reports [47] indicated that only a small amount of *P*-700 underwent a reversible reaction: generally 1–10% of the total amount of *P*-700 was reversible. As shown in Fig. 9, in experiments carried out by the present authors [50], *P*-700 reversibility was observed after dithionite addition, but the total amount of *P*-700 involved in this reversible reaction was never more than 5–10% of the amount undergoing photooxidation when the bound iron-sulfur center was available as an electron acceptor (in the presence of the mild reductant, ascorbate). This quantitative problem has led the present reviewers to a more cautious view of the demonstration of 'X' as an intermediate involved as an electron acceptor prior to the bound iron-sulfur center (for a recent review, see ref. 51).

c. Electrochemical and kinetic studies of *P*-700 photoreactions. A major technical difficulty in studying the low potential electron acceptors of Photosystem I has been the necessity of using alkaline pH (pH 9–11) in the presence of dithionite in order to generate extremely electronegative oxidation-reduction potentials which are necessary to reduce the iron-sulfur centers. Ke et al. [52] have attempted to overcome this problem by use of electrochemical procedures to generate potentials as low as –750 mV. In a recent study of the Photosystem I charge separation at 15 K under controlled potential [52], it was found that the extent of *P*-700 photooxidation did not begin to decrease until an oxidation-reduction potential of approximately –730 mV was reached. In this case, the amount of *P*-700 photooxidized was identical to that detected at higher potentials, indicating that almost all of the *P*-700 pool was being observed. Since these potentials are well below those reported for the bound iron-sulfur centers, Ke and co-workers concluded an additional electron acceptor must function when the iron-sulfur centers are reduced by accepting an electron in the Photosystem I primary reaction. Consistent with this view was the observation that the reversibility of the *P*-700 change at 15 K increased as the iron-sulfur centers were reduced in the dark such that at potentials below approximately –650 mV, $P\text{-}700^+$ decay was totally reversed after the cessation of illumination.

In the same report, Ke et al. [52] also found that when *P*-700 photooxidation as a function of ambient potential was measured at approximately 77 K instead of at 15 K, the ability to photooxidize *P*-700 was lost as a component with a midpoint potential of –530 mV was reduced in the dark. This midpoint is almost identical to that of the photoreducible iron-sulfur center (Center A) and is also the same value obtained by Lozier and Butler (ref. 24, also see ref. 53) in their study of *P*-700 photooxidation at liquid nitrogen temperatures. It is difficult to understand the differences in results reported when measurements of *P*-700 photooxidation are done either at 15 or 77 K. These results imply the rate of the reaction between the reduced intermediate and the oxidized iron-sulfur center must have a strong temperature dependence such that at 77 K, the iron-sulfur center can compete for the electron in the reduced intermediate as efficiently as $P\text{-}700^+$ while at 15 K this is not the case and the back-reaction between the reduced intermediate and oxidized *P*-700 is a more rapid reaction than the transfer of the electron to the iron-sulfur center.

Sauer et al. [54] have reported flash-induced optical studies of *P*-700 which have been interpreted as indicating that two different electron acceptors function prior to iron-sulfur center A. In this work, kinetic components involved in a back-reaction with *P*-700 were detected after laser flash activation of Triton X-100 Photosystem I fragments under various conditions. Under mild reducing conditions, a 30-ms decay due to a back-reaction between $P\text{-}700^+$ and $P\text{-}430^-$ (a component presumed to be a bound iron-sulfur center, see Section IIA-4 and ref. 55) was demonstrated. Under more strongly reducing con-

ditions, a more rapid back-reaction time of 250 μs was observed and a third decay time of 3 μs was detected under the most reducing conditions studied. The 2 rapid decay times have been taken to indicate back-reactions of $P\text{-}700^+$ with reduced electron carriers functioning prior to iron-sulfur Center A. The component involved in the 250-μs decay was suggested to be iron-sulfur center B, based on spectral characteristics, while the component involved in the 3-μs reaction remained uncharacterized. Since the exact oxidation-reduction state of the iron-sulfur centers or Component 'X' was not monitored by EPR spectroscopy prior to flash activation, these conclusions must be considered as tentative at this stage of investigation, but a study combining EPR and optical methods would seem particularly desirable.

The importance of the above described electrochemical and kinetic measurements in terms of our present understanding of the Photosystem I charge separation should not be underestimated. It is hoped that future studies will monitor the EPR changes of Component 'X' and the bound iron-sulfur centers as well as those of *P*-700 in order to test the role of these carriers in the charge separation of Photosystem I and that a correlation with kinetically observed components can be obtained.

IIA-4. Relation of bound iron-sulfur centers to P-430 and a solubilized iron-sulfur protein. At approximately the same time as membrane-bound iron-sulfur centers were discovered in chloroplasts using EPR spectroscopy, Hiyama and Ke [55] reported an absorbance change in the 430-nm region in Photosystem I fragments which was assigned to a component designated *P*-430. The various properties and characteristics of *P*-430 which led to its assignment as the Photosystem I primary electron acceptor have been reviewed in detail by Ke [56] and only more recent results concerning *P*-430 will be discussed at this time.

Initial studies by Ke and Beinert [57] suggested the equivalence of *P*-430 and a bound iron-sulfur center in Photosystem I. This assignment has been strengthened by recent spectral findings of Shuvalov [58]. In addition to absorption bands at 420 and 445 nm, Shuvalov detected a weaker absorption band at 717 nm, and the latter is similar to an absorption band in this region found for soluble chloroplast ferredoxin [59]. Although there was some initial concern that the extinction coefficient for the *P*-430 absorbance change was different from that of soluble chloroplast ferredoxin (see refs. 54 and 55), there is no certainty that the bound iron-sulfur centers are of the same chemical nature as the center in chloroplast ferredoxin. If the bound centers actually contain 4 iron atoms per center, the absorbance changes identified with *P*-430 are qualitatively similar to those of soluble 4-iron ferredoxins. The relationship of *P*-430 to the 2 different iron-sulfur centers in Photosystem I has not yet been clarified, and it is not clear if the absorbance change correlates with only one or with both of the centers.

Shuvalov [58] was also able to detect delayed luminescence which originated from Photosystem I. Characterization of this reaction indicated that the luminescence arose as a consequence of a back-reaction between $P\text{-}700^+$ and $P\text{-}430^-$. This additional feature of the Photosystem I charge separation event should allow for more detailed analyses of the relationship of *P*-430 to the bound iron-sulfur centers by correlation of the properties of the delayed luminescence reaction with the reactions of the bound iron-sulfur centers. The role of Component 'X' in these reactions should also be a subject for further investigation.

The only iron-sulfur protein solubilized from chloroplast membranes has been found to contain 4 atoms of iron per mol of protein [60]. This protein, on reduction with dithionite, shows a broad decrease in absorbance in the 430-nm region, reminiscent of the *P*-430 absorbance change detected on photoreduction by Hiyama and Ke. However, the

liquid helium range EPR spectrum of the reduced isolated protein did not resemble either Photosystem I iron-sulfur center (A or B), and it was impossible to associate this protein with any in vivo center. The isolated protein, which had an electronegative midpoint oxidation-reduction potential (approximately –450 mV, Malkin, R. and Aparicio, P.J., unpublished observations), was distinguishable from soluble chloroplast ferredoxin so that the likelihood that the bound centers were merely of the soluble ferredoxin type is remote. Until more mild extraction procedures can be used to obtain this protein in a more 'native' state, it will be difficult to relate it to *P*-430 or to the bound iron-sulfur centers.

IIA-5. Hypotheses concerning the function of bound iron-sulfur centers in the Photosystem I reaction center complex. Several proposals have been put forward concerning the role of the bound iron-sulfur centers in the primary photochemical reaction of Photosytem I. Early ideas, based primarily on the low-temperature photoreducibility of Center A, suggested that this center was the Photosystem I primary electron acceptor [8–10]. As has been discussed in detail, this terminology may not be appropriate in a strict sense because of the possible existence of intermediates which undergo photoreduction prior to the bound iron-sulfur center.

More recent ideas on the role of the iron-sulfur centers have assigned them as secondary electron acceptors within the Photosystem I primary electron acceptor complex. Evans et al. [45] indicated electron transfer first occurs to Component 'X' and then this carrier can pass its electron to either Center A or Center B (Fig. 11A). Electron transfer from Center B to Center A could also occur according to this model, but reduction of soluble chloroplast ferredoxin only involved Center A. A more recent report by Evans and co-workers [31] (Fig. 11B) has presented a newer proposal in which Centers B and A function in a linear sequence and Center A again interacts with soluble ferredoxin. Both these models take into account the close association of the two iron-sulfur centers indicating they could be accomodated in a single protein which would contain both centers.

Bolton [49] has suggested a different function for the different iron-sulfur centers (Fig. 11C) in which Center A is involved in noncyclic electron transport to NADP while Center B is involved in a cyclic transfer of electrons around Photosystem I. This arrangement suggests that Centers A and B exist in discrete proteins which are probably located in different sites in the chloroplast membrane although both would be in close proximity to Component 'X'. The recent proposal of Arnon et al. [15] has some similarities to that of Bolton in that it proposes that the bound iron-sulfur centers are functioning in different electron transfer processes. As has been previously pointed out, however, the conclusions in the latter case are not based on a correct interpretation of the EPR data and the results can also be explained in terms of both Center A and B interacting with soluble ferredoxin and NADP regardless of the electron donor system used. The results do appear to indicate that both Centers A and B may be involved in noncyclic electron transfer to NADP.

The differences in the above hypotheses deal mainly with the relationship of the two different iron-sulfur centers. A direct electron transfer from Center B to A or vice-versa has never been demonstrated although evidence does indicate that both centers can interact with the same physiological electron acceptor system, ferredoxin plus NADP. The problem of the role of Center B in relation to its ability to be photoreduced at cryogenic temperatures is central to these models. This question has become a subject of some controversy as various reports have recently appeared which present conflicting data on this point. According to the model in Fig. 11B, Center B is obligately required for the photo-

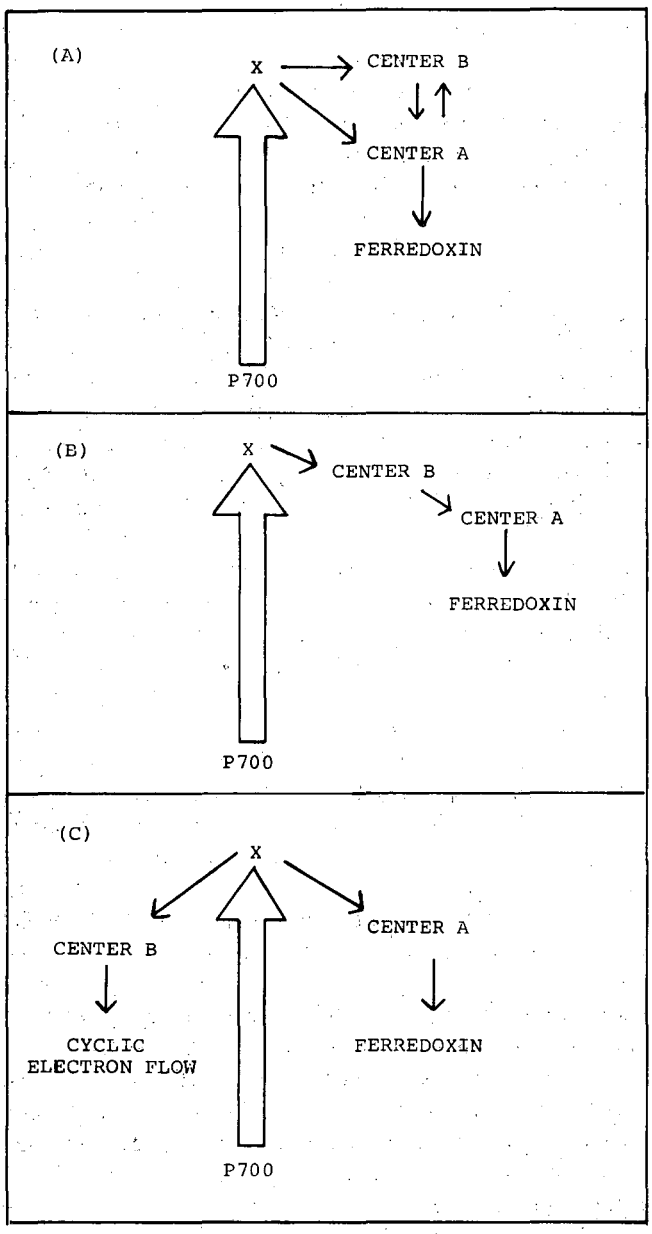

Fig. 11. Models for the site of function of components in the primary electron acceptor complex of chloroplast Photosystem I. (A) from ref. 45 (B) from ref. 31 (C) from ref. 49.

reduction of Center A and the reversibility of the reaction between *P*-700$^+$ and X$^-$ should only occur when Center B is reduced prior to low-temperature illumination. Evans et al. [31] in a reinvestigation of this problem found that *P*-700 reversibility appeared at cryogenic temperature when a component with $E_m = -585$ mV was reduced in the dark prior to illumination. This midpoint potential corresponds to that of Center B and a correlation was observed between *P*-700 reversibility and the dark chemical reduction of Center B. In

experiments using optical methods to follow the reactions of *P*-700 at cryogenic temperatures, Demeter and Ke [61] found that *P*-700 reversibility correlated with a component having a midpoint oxidation-reduction potential of −530 mV; this value would be consistent with the reported midpoint potential of iron-sulfur Center A. Thus, these two reports present results which are contradictory. It should be pointed out that there are many technical complications in these types of measurements which make them difficult to accomplish. In the case of the EPR studies of the *P*-700 reversibility, the presence of redox mediators, which are essential for redox equilibrium to be attained between added reductant and bound carriers, results in there being large free-radical signals in the $g = 2.00$ region where the $P\text{-}700^+$ free radical is detected. Thus, the relative amount of the mediators which are added is critical so that a large background signal is not present. Optical methods, which can be used to monitor *P*-700 without interference from such mediators, suffer from the disadvantage that simultaneous monitoring of the states of the iron-sulfur centers is not possible. In addition, the optical measurements are generally done in a medium which contains high concentrations of glycerol in order to produce optically clear glasses, and the effect of this medium on redox equilibrium has not been studied in detail.

Although some workers believe Center B must function prior to Center A in a linear chain, based on its more electronegative E_m, it should be stressed that redox measurements are made under equilibrium conditions and that they do not reflect a situation which exists during continuous illumination i.e. in the steady state. In addition, the evidence that Center A undergoes photoreduction at cryogenic temperatures in a quantitative reaction with *P*-700 is beyond dispute, while the reactions of Center B at cryogenic temperatures, as described above, are less well defined at this time. Clearly, no definitive answer can yet be given as to the exact site of function of these carriers; this is an area of investigation currently being pursued in several laboratories, and it is anticipated these problems will be resolved in the near future.

IIB. The 'Rieske' iron-sulfur center

IIB-1. EPR and oxidation-reduction properties. In 1975, Malkin and Aparicio identified a third chloroplast iron-sulfur center [62] which was characterized by the appearance of the following absorptions on reduction: $g_z = 2.02$, $g_y = 1.89$ and $g_x = 1.78$. Because of

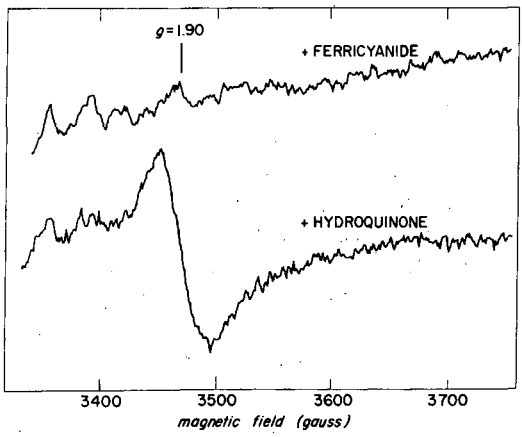

Fig. 12. EPR spectrum of the 'Rieske' iron-sulfur center in spinach chloroplasts. From ref. 62.

overlapping signals from other paramagnetic components present in chloroplasts, this center has routinely been characterized based on the $g_y = 1.89$ signal; it has commonly been referred to as the '$g = 1.89$' iron-sulfur center (Fig. 12).

The EPR properties and the oxidation-reduction characteristics of the $g = 1.89$ iron-sulfur center are similar to those of a center first found by Rieske and co-workers in mitochondria and submitochondrial fragments [63,64]. Hence, this type of center has also been referred to as the 'Rieske' $g = 1.89$ iron-sulfur center. Studies of substrate reduction in the presence and absence of inhibitors have shown that this center has a site of function near cytochrome c_1 in the mitochondria. Consistent with this role is the presence of the 'Rieske' center in mitochondrial Complex III (for a recent review, see ref. 65).

The 'Rieske' center in chloroplasts is reduced by mild reductants, such as hydroquinone (see Fig. 12), and has been found to have an $E_m = +290$ mV [62]. This value is very similar to that of the mitochondrial center and to 'Rieske' centers in chromatophores from several photosynthetic bacteria (cf. Section IIIA). The midpoint potential of the chloroplast center was found to be independent of pH from 6.0 to 8.0 [62] but, following the demonstration of a pK for this center in other systems (see below), the dependence on pH of the chloroplast center should be reinvestigated over a wider pH range.

IIB-2. Site of function. The site of function of the 'Rieske' center in the chloroplast electron transfer chain is not yet known with certainty. Because of the reported E_m and its similarity to its mitochondrial counterpart, it might be expected to function in the region of the chloroplast c-type cytochrome, cytochrome f. A complex containing cytochromes b_6 and f has been isolated from chloroplasts by Nelson and Neumann [66], and they have suggested this complex is analogous to mitochondrial Complex III and therefore might contain an iron-sulfur center, presumably the chloroplast 'Rieske' iron-sulfur center. We have been unable to detect the 'Rieske' center in this complex after EPR analysis at cryogenic temperatures. Our preparations of the cytochrome complex, although containing non-heme iron as does the original preparation of Nelson and Neumann, do not contain significant amounts of acid-labile sulfide. It appears that the non-heme iron may be a contaminant in the preparation and that the 'Rieske' center is not a component of the isolated complex or that it may be lost from the complex during the preparative procedure. Evidence has recently been presented that the 'Rieske' center can be lost from preparations of mitochondrial Complex III made with the detergent, Triton X-100 [67, 68], and this detergent is used in the procedure of Nelson and Neumann to remove chlorophyll from the cytochrome complex.

Two recent findings are consistent with the 'Rieske' center functioning in the chloroplast electron transfer chain between plastoquinone and cytochrome f [69,70]. As shown in Fig. 13, it is possible to reduce the 'Rieske' center in the dark with the electron donor, duroquinol, and this reduction is sensitive to the plastoquinone antagonist, dibromothymoquinone but not to 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) [69]. Electron transfer from duroquinol to the electron acceptor oxygen is also sensitive to dibromothymoquinone but not to DCMU. DCMU is widely accepted to inhibit chloroplast electron transfer reactions at a site on the reducing side of the plastoquinone pool [71] and dibromothymoquinone has been shown to inhibit on the oxidizing side of plastoquinone [72]. These results indicate duroquinol donates electrons through the plastoquinone pool to reduce the 'Rieske' center in a dibromothymoquinone-sensitive reaction.

Cytochrome f is also reduced by duroquinol in the dark in a dibromothymoquinone-sensitive reaction [69]. Because the E_m of cytochrome f is considerably more positive

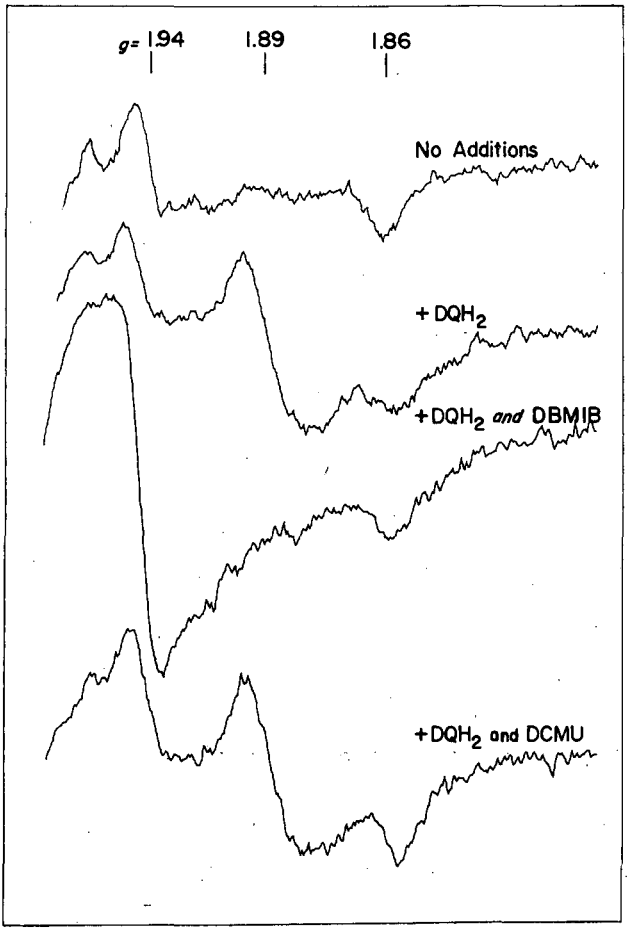

Fig. 13. Reduction of the 'Rieske' iron-sulfur center in chloroplasts by duroquinol in the dark and the effect of noncyclic electron transfer inhibitors. From ref. 69. DQH_2, duroquinol; DBMIB, dibromothymoquinone.

than that of the 'Rieske' center (estimates give values from +350 to +380 mV, see refs. 73–75), one would predict that the 'Rieske' center functions on the reducing side of cytochrome *f*, as shown in Eqn. 1:

Plastoquinone → 'Rieske' center → cytochrome *f* (1)

The localization of the 'Rieske' center between plastoquinone and cytochrome *f* has been confirmed in studies with a mutant of *Lemna* (duckweed) [70]. Previous studies of partial electron transfer reactions in this mutant by Shahak et al. [76] indicated a block between plastoquinone and cytochrome *f*. Most significantly, the chloroplasts from the mutant were able to photooxidize cytochrome *f* but could not reduce the carrier even though electron transfer from water to the plastoquinone pool was unaffected. As shown in Fig. 14, EPR analysis of chloroplasts from wild-type *Lemna* show, on reduction, the $g = 1.89$ EPR signal of the 'Rieske' center while chloroplasts from the mutant show no

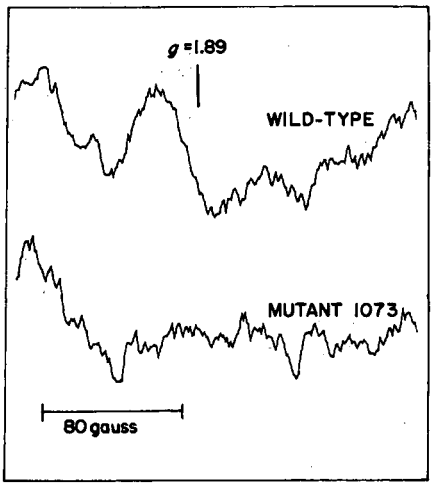

Fig. 14. The $g = 1.89$ EPR signal of the reduced 'Rieske' iron-sulfur center in chloroplasts from wild-type and a mutant of *Lemna*. From ref. 70.

$g = 1.89$ signal on reduction under similar conditions. This analysis indicates the mutational block in the organism most likely involves the 'Rieske' center, again supporting a site of function between plastoquinone and cytochrome *f*.

Consistent with this proposed site are the results of Bering et al. [77] who have studied the chelator sensitivity of chloroplast electron transfer reactions and concluded that a metalloprotein is involved in electron transfer between plastoquinone and cytochrome *f* in the noncyclic electron transport chain. A direct examination of the effect of the inhibitory chelator, bathophenanthroline, on the 'Rieske' center would be particularly informative in relation to these results.

IIC. Developmental studies of bound iron-sulfur centers during greening

The identification of different types of membrane-bound iron-sulfur centers in chloroplasts led to a consideration of the development of such centers during the greening process [78]. In this type of study it is possible to examine the chloroplast precursor, the etioplast, in dark-grown plants for its content of bound iron-sulfur centers and then follow the appearance of such centers as the etioplast develops into a photosynthetically competent chloroplast.

It is well established that a number of different chloroplast electron carriers are present in etioplasts. These include cytochromes [79,80], plastocyanin [80,81], soluble chloroplast ferredoxin [82,83] and the flavoprotein, ferredoxin-NADP reductase [82]. No low-potential iron-sulfur centers were detected when dark-grown barley etioplasts were examined after reduction with dithionite (Fig. 15), indicating the absence of Centers A and B, but the $g = 1.89$ 'Rieske' center was observed after reduction with ascorbate (Fig. 16) [78]. On the basis of cytochrome *f* content of etioplasts and chloroplasts, which does not change during greening, it appears that the amount of the 'Rieske' center is relatively constant over the entire developmental period.

When low-potential iron-sulfur centers were not found in etioplasts, it was expected they would appear during greening in continuous light, and this expectation was verified experimentally. Chemical detection of the low-potential iron-sulfur centers indicated

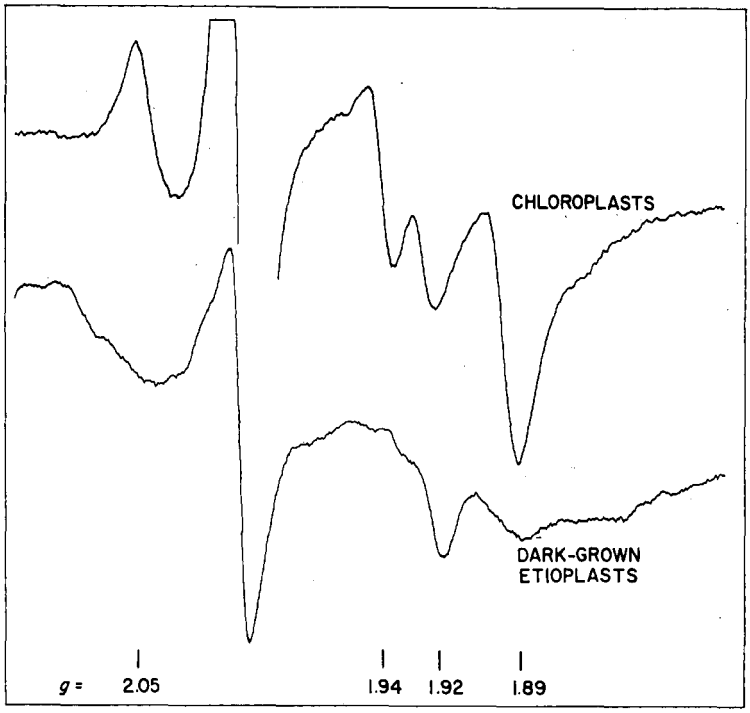

Fig. 15. Low potential iron-sulfur centers in chloroplasts and etioplasts from barley. From ref. 78.

both Center A and B appeared during greening and no kinetic disparity between the appearance of the two centers could be observed [78].

A correlation between the appearance of the photoreducible iron-sulfur center (Center A) with that of the Photosystem I reaction center was also found in this work by measuring the light-induced charge separation at cryogenic temperature [78]. As shown in Fig. 17, both *P*-700 and iron-sulfur Center A show a similar time-course of appearance during greening after a short lag period (about 1–2 h). It was concluded that a stable charge separation in the Photosystem I reaction center complex requires the presence of

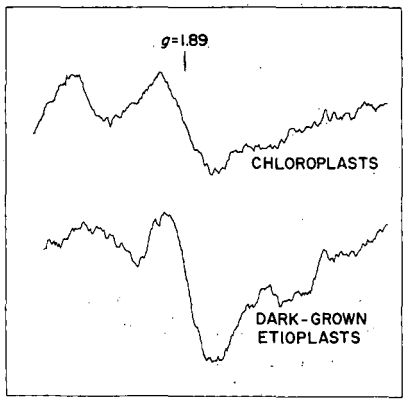

Fig. 16. The 'Rieske' iron-sulfur center in chloroplasts and etioplasts from barley. From ref. 78.

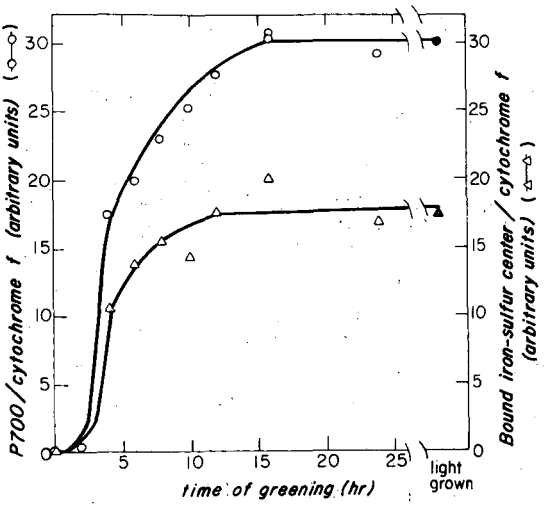

Fig. 17. Time course of the appearance of the Photosystem I reaction center as measured by *P*-700 photooxidation and bound iron-sulfur center photoreduction at 15 K during greening of barley. From ref. 78.

the low-potential iron-sulfur center (Center A) as well as the reaction center chlorophyll, *P*-700.

These studies reveal important characteristics of the assembly of photosynthetically competent Photosystem I reaction centers. It appears that the presence of a bound iron-sulfur center is essential for the formation of the Photosystem I reaction center and that light is involved in the synthesis of this center. The mechanism of the light-dependent synthesis of the iron-sulfur center and the subsequent assembly of the iron-sulfur center into the Photosystem I reaction center complex are areas for future study.

III. Membrane-bound iron-sulfur centers in photosynthetic bacteria

Membrane-bound iron-sulfur centers have been observed by low-temperature EPR analysis of chromatophores from all major classes of photosynthetic bacteria. As in the case of chloroplasts, there is a multiplicity of centers, differing in EPR characteristics as well as in oxidation-reduction properties, and both parameters have been used in the characterization of these centers. More recently, it has been possible to solubilize some of these centers from the chromatophore membranes and to demonstrate their association with specific enzymic activities. In some respects, these centers have properties which are strongly reminiscent of mitochondrial iron-sulfur centers in that they are associated with specific dehydrogenases.

IIIA. The 'Rieske' iron-sulfur center

An EPR signal characteristic of the reduced form of the 'Rieske' iron-sulfur center (g = 1.89) has been observed in chromatophore preparations from several different types of photosynthetic bacteria. This center is present in the green sulfur bacteria [84], the purple sulfur bacteria [85,86], and the purple non-sulfur bacteria [87,88]. The EPR spectrum of the center in chromatophores of *Rhodospirillum rubrum*, shown in Fig. 18, char-

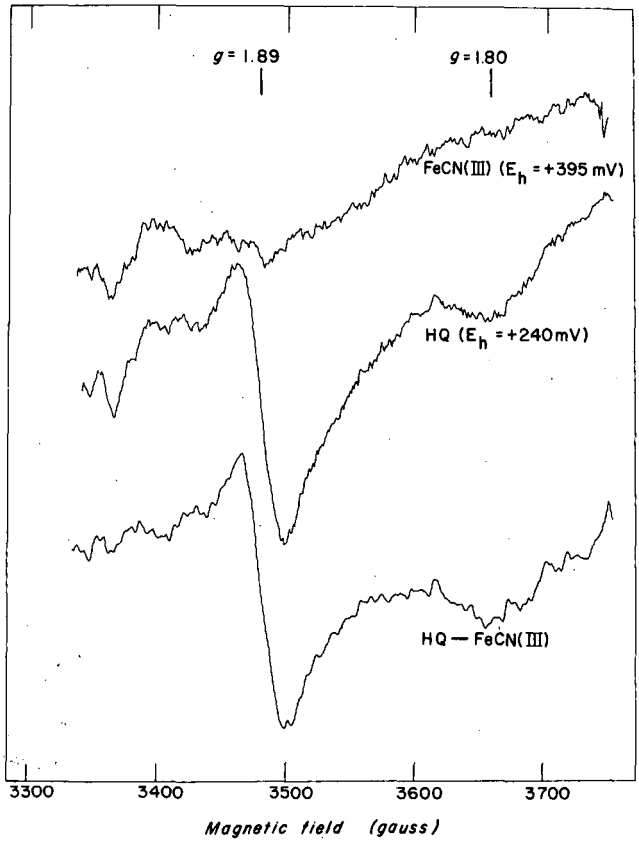

Fig. 18. EPR spectrum of the 'Rieske' iron-sulfur center in chromatophores from *R. rubrum*. HQ, hydroquinone; FeCN(III), ferricyanide.

acterizes this center as a high-potential iron-sulfur center, being oxidized by ferricyanide and reduced by hydroquinone.

Initial reports of the midpoint oxidation-reduction potential of the 'Rieske' center in the purple sulfur and non-sulfur bacteria gave values near those reported for the mitochondrial and chloroplast 'Rieske' centers [65,85–87]. As shown in Table IV, these E_m values ranged from +265 to +310 mV. Another initial observation was that these midpoint potentials were reported to be independent of pH over the pH range from 5 to 8 [85–87].

TABLE IV

OXIDATION-REDUCTION PROPERTIES OF THE $g = 1.89$ 'RIESKE' IRON-SULFUR CENTER IN PHOTOSYNTHETIC BACTERIA

Organism	E_m (mV)	ref.
Chromatium	+285 (pH 8.0)	85, 86
Rhodopseudomonas sphaeroides	+285 (pH 5.8–8.2)	88, 89
Rhodopseudomonas capsulata	+310 (pH 5.8–8.2)	87
Rhodospirillum rubrum	+265 (pH 7.7)	90
Chlorobium	+160 (pH 7.0)	84

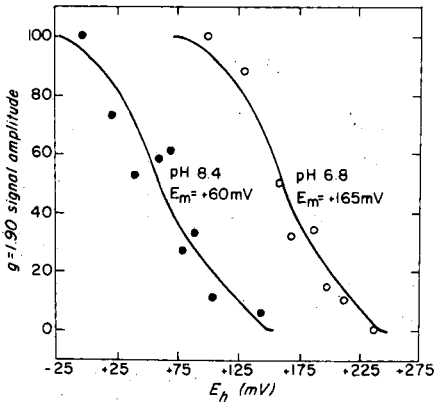

Fig. 19. Oxidation-reduction titrations of the 'Rieske' iron-sulfur center as a function of pH in chromatophores from *Chlorobium*. From ref. 84.

Anomalous results on the 'Rieske' center were subsequently found in chromatophores from the green sulfur bacterium, *Chlorobium* [84]. At pH 7, this center was found to have an E_m = +160 mV, a value nearly 120 mV more reducing than that of this center in any other organism. Even more surprising, the midpoint potential was found to be pH-dependent, as shown in Fig. 19, over the pH range from 6.8 to 8.4. The E_m values over this pH range fit a –60 mV per pH unit dependence and indicated the center takes up one proton as well as one electron on reduction [84].

These results with *Chlorobium* led to a reinvestigation of the pH-dependence of the midpoint potential of the 'Rieske' center in both mitochondria and photosynthetic bacteria [89]. It was found that at an alkaline pH, the E_m of the 'Rieske' center became pH-dependent by –60 mV per pH unit. In mitochondria as well as chromatophores from *Rhodopseudomonas sphaeroides*, a pK of approx. 8 was observed. In the case of *Chromatium*, the 'Rieske' center also shows a pH-dependent midpoint potential at pH values more alkaline than approximately pH 7.5; a pK of about 7.4 has been estimated for this center in this organism (Prince, R.C., Knaff, D.B. and Malkin, R., unpublished observations).

The demonstration of a pH-dependent midpoint potential for the 'Rieske' center and the characteristics of E_m vs. pH-dependence indicates a pK on the oxidized form of the center. At pH values above the pK, the reduction of this center is represented as in Eqn. 2:

$$\text{Fe-S}_{\text{oxidized}} + e^- + \text{H}^+ \rightarrow \text{Fe-S(H)}_{\text{reduced}} \quad (2)$$

At pH values below the pK, the reaction is as shown in Eqn. 3:

$$\text{Fe-S(H}^+)_{\text{oxidized}} + e^- \rightarrow \text{Fe-S(H)}_{\text{reduced}} \quad (3)$$

Although thermodynamic characterization of the 'Rieske' center in photosynthetic bacteria is quite complete, few studies on the function of the center in chromatophore electron transport reactions have been reported. Evans et al. [86] first showed that the 'Rieske' center could undergo photooxidation at physiological temperatures in *Chromatium* chromatophores in the absence of an electron donor and that when an electron donor system, such as reduced dichlorophenolindophenol, was present, the center remained reduced in the light. Similarly, in *Rhodopseudomonas sphaeroides* chromatophores a photooxidation of the center has been demonstrated after steady-state illumina-

tion [88]. However, these experiments, which are on a relatively slow time scale, do not provide an accurate evaluation of the role of the center in bacterial electron transfer processes.

IIIB. g = 1.94 iron-sulfur centers

A multiplicity of iron-sulfur centers with g values of approx. 1.94 in the reduced state have been observed in all photosynthetic bacteria. These centers have been most readily

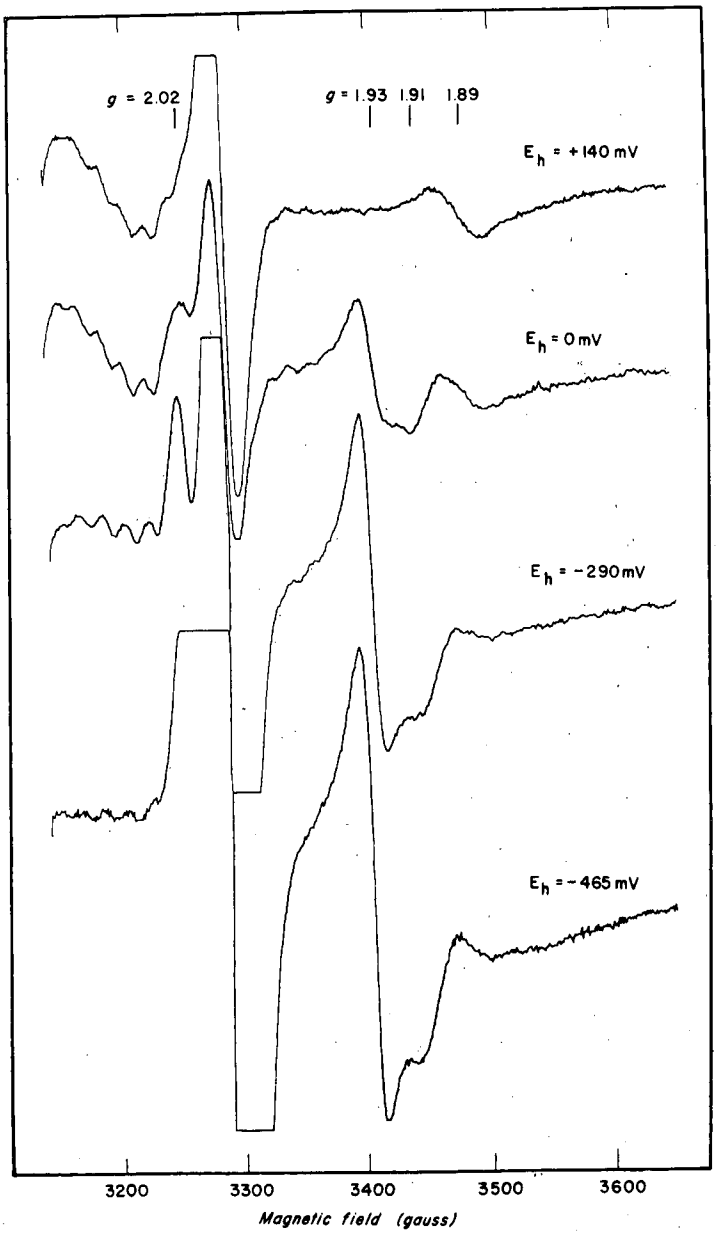

Fig. 20. EPR spectra of membrane-bound $g = 1.93$ iron-sulfur centers in chromatophores from *R. rubrum* at defined oxidation-reduction potentials. From ref. 90.

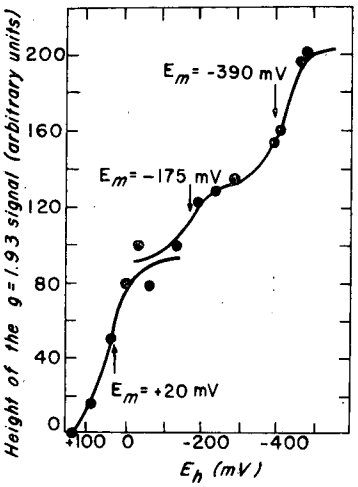

Fig. 21. Oxidation-reduction titration of the membrane-bound $g = 1.93$ iron-sulfur centers in chromatophores from *R. rubrum*. From ref. 90.

distinguished on the basis of their midpoint oxidation-reduction potentials although the temperature profiles of the EPR signals of the centers in *Chromatium* also indicate differences in the centers [86]. The amplitude of the $g = 1.93$ signal in chromatophores of *R. rubrum* at defined oxidation-reduction potential, shown in Fig. 20, is an example of a typical titration of these centers. The complete titration curve is shown in Fig. 21 and demonstrates that 3 different iron-sulfur centers can be resolved by this technique.

The midpoint oxidation-reduction potentials of the $g = 1.94$ iron-sulfur centers in the different photosynthetic bacteria are summarized in Table V. Three centers have been detected in chromatophores of the purple non-sulfur bacteria [87,88,90]; their properties are similar in all the organisms examined. In contrast, in the purple sulfur bacterium, *Chromatium* [86], only two centers have been observed. They have a potential range similar to those observed in the purple non-sulfur bacteria, however. In the green sulfur bacterium, *Chlorobium*, three centers have been detected [84]; one of these has an extremely electronegative midpoint oxidation-reduction potential and the other two centers are again in the potential range from about 0 mV to −200 mV. The low potential center in *Chlorobium* appears to be unique among photosynthetic bacteria but it has a midpoint potential similar to that reported for the low potential centers associated with the chloroplast Photosystem I reaction center complex [29,30].

TABLE V

OXIDATION-REDUCTION PROPERTIES OF THE $g = 1.94$ 'RIESKI' IRON-SULFUR CENTERS IN PHOTOSYNTHETIC BACTERIA

Organism	E_m (mV)	ref.
Chlorobium	−25, −175, −550	84
Chromatium	−50, −290	86
Rhodopseudomonas capsulata	+30, −235, −335	87
Rhodopseudomonas sphaeroides	+40, −200, −350	88
Rhodospirillum rubrum	+20, −175, −390	90

IIIC. g = 2.01 iron-sulfur center

Recently, a high-potential iron-sulfur center associated with a $g = 2.01$ EPR signal which is produced on oxidation has been detected in chromatophores from *Rhodopseudodomonas sphaeroides* [91]. The midpoint oxidation-reduction potential of this center was found to be +80 mV at pH 7.0.

IIID. Function of membrane-bound iron-sulfur centers in chromatophores of photosynthetic bacteria

Although there is some controversy in the literature on the role of the membrane-bound iron-sulfur centers in the primary photochemical reaction of the purple bacteria, recent evidence has clearly demonstrated that these centers do not function in the primary photoevent [28,37]. The EPR signal of the primary electron acceptor has been shown to be characterized by g values of 1.82 and 1.68 in the reduced state, and this spectrum is different from that of any previously known iron-sulfur center [28,37]. In addition, analysis of reaction center preparations from *Rps. sphaeroides* indicates one iron atom per reaction center and the absence of acid-labile sulfide [92,93]. Evidence has accumulated that the primary electron acceptor involves an iron-ubiquinone complex in which the ubiquinone group functions as the obligately required acceptor group (see ref. 28 for a recent review). The function of the single iron atom is not known, but these findings do not lend support to the idea of any involvement of an iron-sulfur center.

In the green sulfur bacteria, however, a role for a low-potential iron-sulfur center (E_m of approximately −550 mV) in the primary reaction has been suggested on the basis of studies by Prince and Olson [94] who have found that the midpoint potential of the acceptor in this organism is between −500 and −550 mV. Jennings and Evans [95] have recently reported the photoreduction at 15 K of a component with a g-value of 1.90 and suggested that this component is the stable primary electron acceptor in *Chlorobium*. It is not clear what relationship if any exists between this component and the low-potential bound iron-sulfur center which has been found in this organism since the g-value of 1.90 is significantly different from that reported for the low-potential center by Knaff and Malkin [84]. This recent work has firmly established that the photochemical reaction in *Chlorobium* differs markedly in properties from that of other photosynthetic bacteria and, in fact, has many similarities to the photoreaction of chloroplast Photosystem I. The detailed role of the low-potential iron-sulfur center in the primary reaction of the green sulfur bacteria is an area for future investigation which will be greatly aided by the preparation of reaction center complexes from this organism.

The complement of iron-sulfur centers in photosynthetic bacteria has many similarities to the centers which have been characterized in mitochondria [6,7] although there are also certainly differences in the two cases as well. The analogy of the 'Rieske' center in bacteria to the similar center that functions in mitochondrial Complex III is particularly strong, and the recent results which were discussed indicate this center has a pH-dependent midpoint potential. This finding raises important possibilities for the 'Rieske' center as a proton-transducing agent as well as an electron carrier. Such a role for the 'Rieske' center in mitochondria had been previously proposed [96,97], but the idea seemed untenable when the center was first reported not to have a pH-dependent midpoint potential. Since reinvestigation of the problem in several systems has now demonstrated a pH-dependent midpoint potential for the 'Rieske' center, the question of its possible role as a physiological proton carrier should again be considered.

The $g = 1.94$ iron-sulfur centers and the $g = 2.01$ center have strong resemblances to the corresponding mitochondrial centers. In the latter case, these centers have been shown to be associated with succinic or NADH dehydrogenases [6,7]. An association of three of these centers with the bacterial succinic dehydrogenase of *Rps. sphaeroides* was first shown by Ingledew and Prince [91] when it was found that alkaline treatment of chromatophores resulted in the solubilization of a succinic dehydrogenase, and that the enzyme contained 3 different iron-sulfur centers: the high-potential $g = 2.01$ center and 2 $g = 1.94$ centers ($E_m = +50$ and -250 mV). Thus, the bacterial enzyme had an iron-sulfur composition almost identical to that of solubilized mitochondrial succinic dehydrogenase which is known to contain three iron-sulfur centers (designated S-1, S-2 and S-3 see refs. 6 and 7). The dehydrogenase-depleted chromatophores retained some residual $g = 1.94$ EPR signal, and Ingledew and Prince speculated that this third $g = 1.94$ center could be associated with the bacterial NADH dehydrogenase, but conclusive evidence on this point was not presented.

The solubilization and characterization of a succinic dehydrogenase from *R. rubrum* by means of treatment of chromatophores with detergents also demonstrates that at least some of the membrane-bound iron-sulfur centers in this organism are associated with succinic dehydrogenase [90]. In this case, the soluble enzyme contained four different iron-sulfur centers. Three of these centers had $g = 1.94$ signals on reduction and their midpoint potentials were +50, -160 and -380 mV. The fourth center had a $g = 2.01$ EPR signal in the oxidized state, but no midpoint potential was reported. The presence of a -380 mV component in this purified enzyme raises the possibility that the enzyme is similar to that characterized by Ohnishi et al. [98] from mitochondria in which a -400 mV iron-sulfur center was observed. In the latter case, it appears that the -250 mV iron-sulfur center found in the enzyme can be altered and converted to the -400 mV form in a reversible manner since rebinding of the soluble enzyme to mitochondrial Complex III caused a shift in the potential of the center back to -250 mV. The experiments with the bacterial enzyme indicate the complexity of the enzyme from this source and at this stage of investigation it is difficult to relate the centers in the purified enzyme with those observed in situ. It is possible that the centers in the soluble enzyme are heterogeneous in that modification during extraction and purification may have occurred. However, the availability of these purified succinic dehydrogenases should be useful in further characterization of the bound iron-sulfur centers in these organisms.

IV. Concluding remarks

While early studies of iron-sulfur proteins resulted in the characterization of soluble ferredoxin-type proteins from different sources and an understanding of the function of these proteins in various cellular reactions, recent emphasis has been placed on the function of iron-sulfur centers in more complex membranous subcellular organelles, such as the mitochondrion, the bacterial chromatophore and the chloroplast. The results of the past 6 years which we have tried to summarize in this review indicate that the membrane-bound iron-sulfur centers are involved in a number of different types of electron transfer processes in photosynthetic systems. The exact site of function of all these carriers is not yet known, and it is anticipated that the next few years will yield results which will answer some of the questions which have been posed about the structure and function of the membrane-bound iron-sulfur centers in photosynthetic organisms.

Acknowledgements

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