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FLASH PHOTOLYSIS ELECTRON SPIN RESONANCE STUDIES OF THE ELECTRON ACCEPTOR SPECIES AT LOW TEMPERATURES IN PHOTOSYSTEM I OF SPINACH SUBCHLOROPLAST PARTICLES*

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SUMMARY

The light-induced electron spin resonance signals of Photosystem I spinach subchloroplast particles have been studied at approximately 6 °K. Using the technique of flash photolysis–electron spin resonance with actinic illumination at 647 nm, a kinetic analysis of the previously observed bound ferredoxin ESR signals was carried out. Signal I ($P700^+$) exhibits a partial light-reversible behavior at 6 °K so it was expected that if the bound ferredoxin is the primary acceptor of Photosystem I, it should also exhibit a partial reversible behavior. However, none of the bound ferredoxin ESR signals showed any such light reversible behavior. A search to wider fields revealed two components which did exhibit the expected kinetic behavior. These components are very broad (about 80 G) and are centered at $g = 1.75$ and $g = 2.07$. These two components exhibit the expected characteristics of the primary electron acceptor. A model is presented to account for the reversible and irreversible photochemical changes in Photosystem I. The possible identity of the primary acceptor responsible for these two new components, is discussed in terms of the available information. The primary acceptor may be an iron–sulfur protein, but not of the type characteristic of the bound or water-soluble ferredoxins found so far in chloroplasts.

INTRODUCTION

Recently there has been a considerable debate as to the identity of the primary acceptor of Photosystem I in green plant and algal photosynthesis. It is well known that a water-soluble ferredoxin is involved as an electron-transfer component on the reducing side of Photosystem I [1, 2]; however, since membrane fragments enriched in Photosystem I which contain no soluble ferredoxin can still undergo the primary photochemistry of Photosystem I, there must be one or more electron transfer components which precede the soluble ferredoxin.

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In 1971 Malkin and Bearden [3] showed that spinach chloroplasts, when illuminated with red light at approximately 77 °K, exhibit a new electron spin resonauce (ESR) signal at 25 °K. Since this new component is formed in parallel with the formation of Signal I ($P700^+$), they suggested that this new component is a ferredoxin which functions as the primary acceptor of Photosystem I. This proposal was given further support by quantitative intensity comparisons and the fact that the production of this component is possible with far-red light ($\lambda > 700$ nm) [4–7]. Evans et al. [6] detected two ferredoxin-like ESR signals, both of which could be photoproduced, and suggested that the two components might be two active centers of the same protein.

Ke [8–10] has suggested that an optical absorbing component P430 arises from the primary acceptor of Photosystem I. On the basis of similarity of the light-minus-dark optical spectra he assigns this optical component to a bound ferredoxin. He has determined that the redox potential of P430 is −470 mV [9].

More recently Ke et al. [11] have measured the intensities of the ferredoxin ESR components as a function of redox potential. They found three distinct components and claimed that each was produced by a two-electron reduction. However, this finding has been disputed by Evans et al. [12] who claim that only two components are produced each by a one-electron reduction. One component has a redox potential (E_m at pH 10) of −563 mV with g factors of 2.05, 1.94 and 1.86, and the other component has a redox potential (E_m at pH 10) of −604 mV with g factors of 2.05, 1.92 and 1.89. We tend to believe that the results of Evans et al. [12] are more reasonable in view of the fact that only one electron is available for reduction in each reaction center.

The primary acceptor of Photosystem I should exhibit the same formation and decay kinetics as Signal I ($P700^+$) at low temperatures if we assume that the only decay process is direct return of the electron from the primary acceptor to $P700^+$. It has been known for some time that P700 exhibits a partial reversible photooxidation at temperatures of approximately 77 °K [13]. We have determined that this reversible component of P700 has a decay half life of about 0.8 s which is temperature independent from 5 to approx. 150 °K [14]. The primary acceptor should also exhibit this decay behavior and thus we initiated a study of the kinetics of each of the ferredoxin ESR components of Photosystem I using the flash photolysis electron spin resonance technique. The results of this study are presented in this paper.

MATERIALS AND METHODS

Spinach subchloroplast particles were prepared according to the method of Vernon et al. [15, 16] with the detergent Triton X-100. The Photosystem I particles were suspended in a 0.05 M phosphate buffer (pH 7.7) with 0.5 M sucrose, 0.01 M NaCl and 50 % glycerol*. The particles were stored in the dark at −20 °C prior to use.

The actinic illumination was a laser beam operated at 647.1 nm interrupted with a programable electronic shutter as described earlier [14]. The ESR signals

* The glycerol is added so that the preparation will form a glass at low temperatures. The glycerol has no effect on the kinetic behavior of these particles at low temperatures although it does enhance the proportion of Signal I which is reversible.

were accumulated on a computer of averaged transients [14]. Low temperatures were maintained with an Air Products Model LTD-3-110 "Helitran" liquid He dewar and transfer system.

RESULTS

The frozen-in ESR signal at 6 °K for the Triton subchloroplast particles is shown in Fig. 1. Little or no ESR signal is seen for samples frozen in the dark prior to illumination. It appears that both components of Evans et al. [12] are formed because the g factors we obtain are in good agreement with theirs. The only exception to this good agreement appears to be an absorption at $g = 2.06$, but we found that this small intensity absorption was not reproducible in all samples.

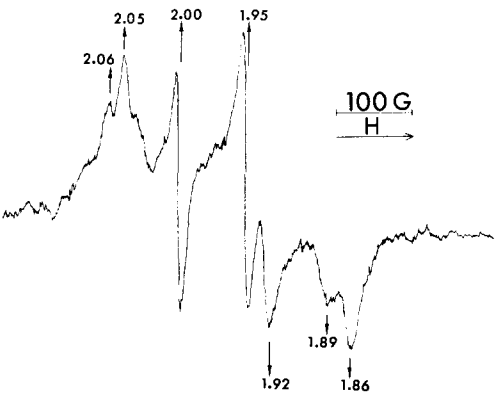

Fig. 1. ESR spectrum frozen in at 6 °K after irradiation at 647 nm of Triton Photosystem I subchloroplast particles in 0.05 M phosphate buffer (pH 7.7) with 0.5 M sucrose, 0.01 M NaCl and 50 % glycerol. Modulation amplitude 12 G; microwave power 40 mW. Under these conditions, Signal I at $g = 2.00$ is highly saturated.

The Triton subchloroplast samples exhibited a light reversible behavior for Signal I which was often about 1 % of the total frozen-in Signal I. This small percentage could be increased to about 5 % by allowing the samples to be frozen slowly down to 6 °K.

An intensive search was made for light reversible behavior of each of the components of the two bound ferredoxin ESR signals. However, this search was unsuccessful. We believe that our signal-to-noise ratio is such that we can, on the basis of these results, virtually rule out the two bound ferredoxin components as candidates for the primary acceptor of Photosystem I.

We then began a search outside the range of most ferredoxin ESR signals. To higher field at $g \approx 1.75$, we found an absorption which did exhibit the same reversible characteristics of Signal I. It can only be detected with a high microwave power of 200 mW and with high gain and $\approx$ 20 G modulation amplitude for the ESR operating conditions. This component has a linewidth of at least 80 G and an absorption-like shape in the negative direction. To lower field, at $g \approx 2.07$, we found another component which also has a large linewidth (at least 80 G) and an absorption-

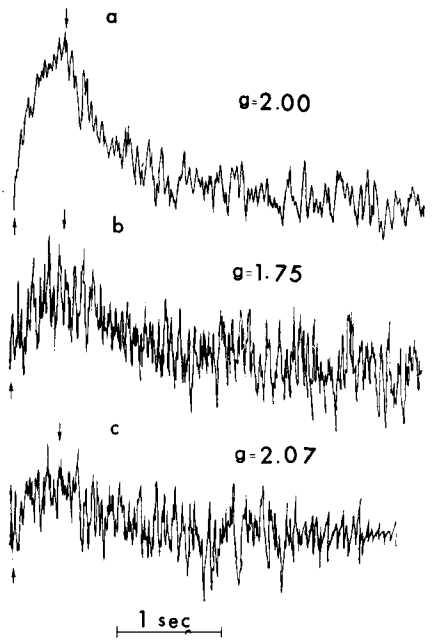

Fig. 2. Time course of the formation and decay of ESR signals at 6 °K during flash photolysis at 647 nm of Triton Photosystem I subchloroplast particles in 0.05 M phosphate buffer (pH 7.7) with 0.5 M sucrose, 0.01 M NaCl and 50 % glycerol. (a) The high field peak position of Signal I at 1 mW power and 5 G modulation amplitude. (b) The $g = 1.75$ field position at 200 mW power and 25 G modulation amplitude. The display is reversed in phase from the actual signal. (c) The $g = 2.07$ field position at 200 mW power and 25 G modulation amplitude.

like shape but in the positive direction. These components could be the g_{xx} and g_{zz} lines of an $S = \frac{1}{2}$ system with rhombic symmetry [17]. It can be seen from Fig. 2 that these two components exhibit the same kinetic behavior as Signal I as is expected for the primary acceptor. It should be noted that the signal-to-noise ratio for Signal I is approximately 200 times that of the weak $g = 1.75$ and 2.07 components when we consider the relative power levels, the modulation amplitudes, and the spectrometer gain operating conditions. Each of the kinetic curves of Fig. 2 was obtained by the accumulation of 256 scans by the instrument computer, and it is evident from the figure that the signal-to-noise conditions for Signal I are much more favorable than those of the other components.

We are certain that these new signals are not due to an artifact of heating by the light flash as no kinetic signal is seen on any of the ferredoxin peaks under similar operating conditions. As well we are convinced that there were no anomalous power saturation or rapid passage effects observed for these signals. It was observed that the ferredoxin components exhibited no power-saturation effects up to 100 mW power and only a little power saturation at 200 mW. We also looked for rapid passage effects [18], but we found none using the technique suggested to us (Taylor, C. P. S., personal communication).

Since the new $g = 2.07$ and 1.75 components appear in reverse phase to each other, we would expect a derivative-like signal somewhere between these two com-

ponents if these signals arise from an $S = \frac{1}{2}$ state which has rhombic symmetry [17]. We have searched for such a component, but as yet, we have not found one. The signal may well be too broad to be detected or the reversible component may really have axial symmetry in which the 2.07 peak is $g_{\|}$ and the 1.75 peak is $g_{\perp}$.

DISCUSSION

It is clear that these new ESR signals are strong candidates for components of the ESR spectrum of the primary acceptor of Photosystem I. As these g factors are considerably outside the range of known g factors for ferredoxins, it is not likely that the primary acceptor (which we will call X) is a normal ferredoxin although it very well could be an iron–sulfur protein [19]. For instance, a 4-methoxybenzoate *o*-demethylase from *Pseudomonas putida* has g values of 2.01, 1.91 and 1.78 [20].

On the basis of our results we propose the following model to explain the fact that both reversible and irreversible photochemical processes occur at low temperatures. We assume that the extent of damage in reaction centers is varied for any given sample:

We assume that some of the reaction centers are damaged, either in sample preparation or in freezing, such that the electron from P700 can reach only the primary acceptor X.

$$\text{P700–X} \not\leftarrow \text{Y}_1\text{–Y}_2 \xrightarrow{h\nu} \text{P700}^+\text{–X}^- \not\leftarrow \text{Y}_1\text{–Y}_2$$

The electron X^- may now return to $P700^+$ via a quantum-mechanical tunneling process [14]. Support for this is found in the fact that the reversible portion of Signal I varies from 1–50 % depending on the sample or species from which the sample was prepared [14].

For other reaction centers in any given sample, the system is not damaged as much; and the electron can reach the secondary component Y_1.

$$\begin{array}{l} \text{P700–X–Y}_1 \not\leftarrow \text{Y}_2 \xrightarrow{h\nu} \text{P700}^+\text{–X}^-\text{–Y}_1 \not\leftarrow \text{Y}_2 \\ \qquad\qquad\qquad\qquad\quad \downarrow \\ \qquad\qquad\qquad\qquad \text{P700}^+\text{–X–Y}_1^- \not\leftarrow \text{Y}_2 \end{array}$$

This process is irreversible once it occurs and accounts for the observation of one of the ferredoxin components*.

Finally, for other reaction centers in a given sample, the system is still less damaged such that the electron can reach component Y_2:

$$\begin{array}{l} \text{P700–X–Y}_1\text{–Y}_2 \xrightarrow{h\nu} \text{P700}^+\text{–X}^-\text{–Y}_1\text{–Y}_2 \\ \qquad\qquad\qquad\qquad\quad \downarrow \\ \qquad\qquad\qquad\qquad \text{P700}^+\text{–X–Y}_1^-\text{–Y}_2 \\ \qquad\qquad\qquad\qquad\quad \downarrow \\ \qquad\qquad\qquad\qquad \text{P700}^+\text{–X–Y}_1\text{–Y}_2^- \end{array}$$

This would account for the irreversible formation of the other ferredoxin component.

An alternative explanation for the irreversible formation of components Y_1 and Y_2 is a parallel scheme in which either Y_1 or Y_2 can be reduced by X^-

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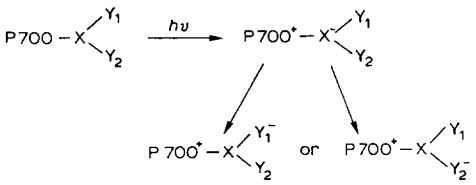
* Component Y_1 is probably the one with the more negative redox potential and therefore is to be compared with component B of Evans et al. [12].



Lozier and Butler [21] have determined the redox potential of −0.53 V for the disappearance of the photo-induced $P700^+$ ESR signal. They claimed this to be the redox potential of the primary acceptor, but, of course, if the electron can return from the primary acceptor at ≈ 77 °K then they were not determining the redox potential of X but of Y_1 or Y_2. Clearly the redox potential of X must be more negative than that of either Y_1 or Y_2.

An enticing possibility for the identity of X would be a reaction-center protein similar to the one found in photosynthetic bacteria [22]. Dutton and Leigh [23, 24] have described a 'photoredoxin' component in this protein which exhibits all the characteristics of a primary acceptor for bacterial photosynthesis. This 'photoredoxin' species has light reversible components at $g = 1.82$ and $g = 1.68$. The 1.68 component is similar to our 1.75 component but as yet, we have found nothing around $g = 1.82$ in our Photosystem I particles.

CONCLUSIONS

It would appear from our results that the ferredoxin components which have been proposed as the primary acceptor of Photosystem I, behave like secondary acceptors and that a new ESR signal with g factors of 1.75 and 2.07 can be considered as the primary acceptor X. Although X may be an iron–sulfur protein, it does not have g factors in the range of normal ferredoxins. Further experiments are in progress, such as a potentiometric titration to characterize the nature of the unknown primary acceptor we have observed, and will be reported in later communications.

ADDENDUM

We have measured the kinetics of initial formation of the bound ferredoxin and Photosystem I ESR signals at 6 °K from dark-incubated samples. An analysis of the relative quantum yields of production of $P700^+$ vs that of the ferredoxin signals showed that the yield of reduced ferredoxin is about 2.5 times less than the yield of $P700^+$.

REFERENCES

1 Arnon, D. I. (1965) Science 149, 1460–1470
2 Vernon, L. P. and Ke, B. (1966) in The Chlorophylls (Vernon, L. P. and Seely, G. R., eds), pp. 569–609, Academic Press, New York
3 Malkin, R. and Bearden, A. J. (1971) Proc. Natl. Acad. Sci. U.S. 68, 16–19
4 Bearden, A. J. and Malkin, R. (1972) Biochem. Biophys. Res. Commun. 46, 1299-1305;
5 Bearden, A. J. and Malkin, R. (1972) Biochim. Biophys. Acta 283, 456–468
6 Evans, M. C. W., Telfer, A. and Lord, A. V. (1972) Biochim. Biophys. Acta 267, 530–537

7 Yang, C. S. and Blumberg, W. E. (1972) Biochem. Biophys. Res. Commun. 46, 422–428
8 Hiyama, T. and Ke, B. (1971) Proc. Natl. Acad. Sci. U.S. 68, 1010–1013
9 Ke, B. (1972) Arch. Biochem. Biophys. 152, 70–77
10 Ke, B. (1973) Biochim. Biophys. Acta 301, 1–33
11 Ke, B., Hansen, R. E. and Beinert, H. (1973) Proc. Natl. Acad. Sci. U.S. 70, 2941–2945
12 Evans, M. C. W., Reeves, S. G. and Cammack, R. (1974) Biochim. Biophys. Acta, in the press
13 Mayne, B. C. and Rubinstein, D. (1966) Nature 210, 734–735
14 Warden, J. T., Mohanty, P. and Bolton, J. R. (1974) Biochem. Biophys. Res. Commun. 59, 822–838
15 Warden, J. T. and Bolton, J. R. (1974) Photochem. Photobiol. 20, 251–262
16 Vernon, L. P., Shaw, E. R. and Ke, B. (1966) J. Biol. Chem. 241, 4101–4109
17 Swartz, H. M., Bolton, J. R. and Borg, D. C (1972) Biological Applications of ESR, pp. 26–28, Wiley Interscience, New York
18 Mailer, C. and Taylor, C. P. S. (1973) Biochim. Biophys. Acta 322, 195–203
19 Blumberg, W. E. and Peisach, J. (1974) Arch. Biochem Biophys. 162, 502–512
20 Bernhardt, F. H., Ruf, H. H. and Standinger, H. (1971) Hoppe-Seyler's Z. Physiol. Chem. 352, 1091–1099
21 Lozier, R. H. and Butler, W. L. (1974) Biochim. Biophys. Acta 333, 460–464
22 Feher, G. (1971) Photochem. Photobiol. 14, 373–387
23 Dutton, P. L., Leigh, J. S. and Wraight, C. A. (1973) FEBS Lett. 36, 169–173
24 Dutton, P. L., Leigh, J. S. and Reed, D. W. (1973) Biochim. Biophys. Acta 292, 654–664