Biochimica et Biophysica Acta, 430 (1976) 555—559

BBA Report

BBA 41281

ELECTRON SPIN RESONANCE SPECTRUM OF SPECIES "X" WHICH MAY FUNCTION AS THE PRIMARY ELECTRON ACCEPTOR IN PHOTOSYSTEM I OF GREEN PLANT PHOTOSYNTHESIS*

ALAN R. MCINTOSH and JAMES R. BOLTON

Photochemistry Unit, Department of Chemistry, University of Western Ontario, London, Ontario N6A 5B7 (Canada)

(Received January 19th, 1976)

Summary

Purified Photosystem I particles from spinach when reduced with 10 mM dithionite at pH 9 exhibited a 50% light reversible-ESR Signal 1 (P-700$^+$) at about 10 K. It was possible to show by signal-averaging techniques that a light-reversible ESR spectrum concomitant with the reversible Signal 1 can be observed with approximate principal g factors at g = 2.07, g = 1.86 and g = 1.75.

Recently there has been some controversy [1—6] in the literature concerning the identity of the primary electron acceptor of Photosystem I in green plant and algal photosynthesis. The early observations of Malkin and Bearden [1] that an iron-sulfur ferredoxin type ESR signal can be photoproduced in stoichiometric proportion to the oxidized Photosystem I donor P-700$^+$ implicated a bound ferredoxin as the Photosystem I primary acceptor. Subsequent work tended to confirm this interpretation [2, 3, 5]. However, it now appears that there are at least two bound ferredoxin components with very low redox potentials [7, 8]. Also, Warden et al. [9] noted that the kinetics of P-700 oxidation and reduction exhibited a small but significant level of reversibility at cryogenic temperatures. The decay half life of the reversible signal is about 0.8 s using a shuttered laser beam at 647 nm. Subsequently, McIntosh et al. [6] reported that the bound ferredoxin signals lacked any reversible component but they did find resonances at g = 1.75 and g = 2.07 which exhibited reversible kinetics identical to that of P-700$^+$. They proposed that this new signal (at g = 1.75 and 2.07) is due to the primary electron acceptor of Photosystem I. In support of this model, Evans and

*Contribution No. 150 from the Photochemistry Unit, University of Western Ontario, London Ontario, Canada.

Cammack [10] observed that the reduction of the bound ferredoxins by sodium dithionite produces an enhancement of the reversible photooxidation of *P*-700 at low temperatures. In fact, they found that most of the $P\text{-}700^+$ ESR signal is light reversible in contrast to the irreversibly formed ferredoxin ESR signals. In a recent paper, Evans et al. [11] reported that when highly purified Photosystem I subchloroplast particles were treated with sodium dithionite at pH 9 and then frozen, a broad reversible ESR component could be seen at g = 1.76 at 10 K in agreement with the work of McIntosh et al. [6]. Evans et al. [11] also obtained a partial ESR spectrum of this new component which we will call "X".

Nevertheless, other investigators have been unable to demonstrate the low temperature reversible $P\text{-}700^+$ kinetics [4, 12]. In addition, Ke et al. [13] indicate that the kinetics of appearance of $P\text{-}700^+$ and the bound ferredoxin signal are identical at low temperatures. However, the work of Ke et al. was done at 13 K and at higher temperatures with a time constant of 0.25 s for kinetic experiments; but the results of the kinetic experiments were presented on a time scale of the order of minutes; and apparently no signal-averaging techniques were employed. In our experience. some signal averaging has been necessary for the clear observation of the reversible $P\text{-}700^+$ kinetics in the absence of dithionite reduction of the subchloroplast particles.

In this paper we continue our study of the spectral species "X" and report on its complete first-derivative ESR spectrum. We have found this to be a rather difficult task because of the difficult signal-to-noise ratios inherent in the very broad signals which are light reversible. In our hands, a straightforward light versus dark spectrum over a range of 1000 G did not provide sufficient signal amplitude for the broad light-reversible components. We were obliged to resort to signal-averaging techniques which we shall describe.

We have utilized the methods of Evans et al.[11] to obtain highly purified Photosystem I Triton subchloroplast particles after DEAE-cellulose chromatography. These particles (containing 5—10 mg of chlorophyll per ml of suspension) were reduced under nitrogen with a fresh 10 mM sodium dithionite solution at pH 9 for about 30 min at 0°C. The reduced particles were then frozen in the dark. At 10 K these particles exhibited a $P\text{-}700^+$ (Signal 1) reversible signal which was about twice as large under illumination as that in the dark after illumination at a power level of 0.1 mW. Thus, these particles displayed a 50% light-reversible character relative to the dark background after illumination. We determined that the total $P\text{-}700^+$ signal which could be obtained under conditions of dithionite reduction plus illumination was very close to the total light-induced $P\text{-}700^+$ signal obtained from particles with no dithionite treatment and with the same chlorophyll concentration.

To obtain the light minus dark spectrum of "X", we employed a Spectra Physics Model 164-01 CW Krypton laser beam at 647 nm with a power of 20 mW This beam was periodically interrupted by a Vincent Associates Model 300-B "Uniblitz" programmable electronic shutter as described previously [9]. In this work the output from the 100 kHz phase detector of the Varian E12 ESR spectrometer was led to an electronic averaging device which collected and averaged successive 0.5-s "light" and "dark" segments of the ESR output and amplified this output by a factor of 16. A complete light-dark cycle was 4 s

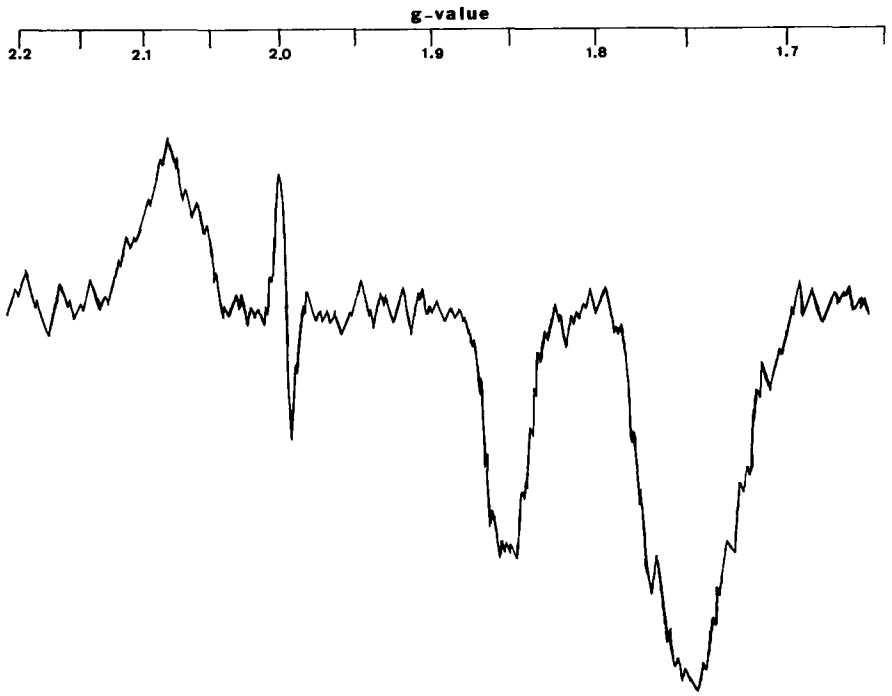

Fig. 1. Light minus dark, first-derivative ESR spectrum recorded at 10 K during flash photolysis at 647 nm of purified Triton Photosystem I particles previously reduced at pH 9 with 10 mM dithionite in 0.05 M Tris with 0.2% Triton X-100. Modulation amplitude = 32 G; microwave power = 100 mW. The vertical axis in the figure is eight times that recorded in the memory of the instrument computer.

long to allow for decay of the light-reversible signals. The difference of the "light"- and "dark"-averaged signals was led to the memory of a Fabri-tek 1072 instrument computer (manufactured by Nicolet Corp). The advantage of this method is that it discriminates against long-term drift of the instrument as the "difference" is computed every 4 s. Several control spectra indicated satisfactory performance of the apparatus. Two 500 G scans at 10 K were made, each requiring 2 h to obtain the complete light minus dark first-derivative ESR spectrum of a purified spinach Photosystem I subchloroplast particle suspension shown in Fig. 1. We had established previously that the subchloroplast preparations were light reversible for many thousands of light and dark cycles. It is to be noted that the $P\text{-}700^+$ signal (Signal 1) at $g = 2.0025$ is highly power saturated and overmodulated under the conditions of the experiment. Fig. 1 clearly shows three g factor components at approximately $g = 2.07$, 1.86 and 1.75. The line shape of the spectrum is not that precisely expected for a first-derivative presentation of a paramagnetic resonance with rhombic g factor symmetry [6].

In the previous work with non-reduced particles [6], it was necessary to accumulate 256 scans on the instrument computer to demonstrate light-reversible signals at $g = 1.75$ and $g = 2.07$. With the purified particles reduced with dithionite the improved signal-to-noise ratio in the light-reversible signal allowed the detection of a good signal in about 16 light-dark cycles with the improved techniques in this present work.

In the model which we have proposed [6] the spectral species "X" functions as the primary acceptor in Photosystem I. Thus, it must have a redox potential more negative than those of the bound ferredoxin components which, in our model, function as secondary acceptors. Consequently, in agreement with Evans et al. [11]. we find that treatment with dithionite, which at pH 9 reduces both bound ferredoxin components, greatly enhances the reversibility of the $P\text{-}700^+$ and X^- signals. The kinetic behavior of the broad signal in Fig. 1 with g factors of 2.07, 1.86 and 1.75 is identical to that of $P\text{-}700^+$. Since, under the conditions of our experiment, the bound ferredoxins are reduced and inactive, the fact that *P*-700 and species "X" are still photoactive provides the best evidence for claiming that species "X" precedes the bound ferredoxins in the electron transport chain on the reducing side of Photosystem I.

As to the identity of species "X" we are puzzled. The g factors and ESR line shape are quite different from those of ferredoxin-type centers although it is almost certain that species "X" contains iron. Iron is probably present because of the large g factor anisotropy observed in the spectrum which could be explained by the proximity of an iron atom or atoms in the molecular complex. We would speculate that Photosystem I is similar to the bacterial photosystem where the presence of iron is well established [19]. If the structure of "X" is similar to the bacterial acceptor, this may explain the unusual line shape. That is, the electron may reduce an organic center such as a quinone; but the proximity of a paramagnetic iron would greatly distort the ESR spectrum. The g factors are similar to those of the primary acceptor in photosynthetic bacteria [14, 15], so a similar structure may prevail although the large difference in redox potential mediates against this hypothesis.

We do not claim to have detected the ultimate primary acceptor of Photosystem I. Studies in the microsecond, nanosecond or even picosecond time range may reveal other components as has been found in bacteria [16—18].

This research was supported by a grant from the National Research Council of Canada.

Addendum

It has come to our attention recently that Evans et al. [20] have reported a similar light-reversible spectrum with g factors of g = 2.08, 1.88 and 1.78 obtained at low temperatures in membrane fractions from the blue-green alga *Chlorogloea fritschii*. Evans et al. were successful in recording this spectrum by using a straightforward light-minus-dark technique.

References

1 Malkin, R. and Bearden, A.J. (1971) Proc. Natl. Acad. Sci. U.S. 68, 16—19.
2 Bearden, A.J. and Malkin, R. (1972) Biochem. Biophys. Res. Commun. 46, 1299—1305.
3 Bearden, A.J. and Malkin, R. (1972) Biochim. Biophys. Acta 283, 456—468.
4 Malkin, R. and Bearden, A.J. (1976) Biochim. Biophys. Acta, in press.
5 Evans, M.C.W., Telfer, A. and Lord, A.V. (1972) Biochim. Biophys. Acta 267, 530—537.
6 McIntosh, A.R., Chu, M. and Bolton, J.R. (1975) Biochim. Biophys. Acta 376, 308—314.
7 Evans, M.C.W., Reeves, S.G. and Cammack, R. (1974) FEBS Lett. 49, 111—114.
8 Ke, B., Hansen, R.E. and Beinert, H. (1973) Proc. Natl. Acad. Sci. U.S. 70, 2941—2945.
9 Warden, J.T., Mohanty, P. and Bolton, J.R. (1974) Biochem. Biophys. Res. Commun. 59, 822—838.
10 Evans, M.C.W. and Cammack, R. (1975) Biochem. Biophys. Res. Commun. 63, 187—193.

11 Evans, M.C.W., Sihra, C.K., Bolton, J.R. and Cammack, R. (1975) Nature 256, 668—670.
12 Visser, J.W.M., Rijgersberg, K.P. and Amesz, J. (1974) Biochim. Biophys. Acta 368, 235—246.
13 Ke, B., Sugahara, K., Shaw, E.R., Hansen, R.E., Hamilton, W.D. and Beinert, H. (1974) Biochim. Biophys. Acta 368, 401—408.
14 Dutton, P.L., Leigh, J.S. and Wraight, C.A. (1973) FEBS Lett. 36, 169—173.
15 Dutton, P.L., Leigh, J.S. and Reed, D.W. (1973) Biochim. Biophys. Acta 292, 654—664.
16 Kaufman, K.J., Dutton, P.L., Netzel, T.L., Leigh, J.S. and Rentzepis, P.M. (1975) Science 188, 1301—1304.
17 Rockley, M.G., Windsor, M.W., Cogdell, R.J. and Parson, W.W. (1975) Proc. Natl. Acad. Sci. U.S. 72, 2251—2255.
18 Fajer, J., Brune, D.C., Davis, M.S., Forman, A. and Spaulding, L.D. (1975) Proc. Natl. Acad. Sci. U.S. 72, 4956—4960.
19 Parson, W.W. and Cogdell, R.J. (1975) Biochim. Biophys. Acta 416, 105—149.
20 Evans, E.H., Cammack, R. and Evans, M.C.W. (1976) Biochem. Biophys. Res. Commun., in press.