

Direct assignment of vitamin K₁ as the secondary acceptor A₁ in photosystem I

(photosynthesis/quinones/electron spin polarization electron paramagnetic resonance/photosystem I reactions)

SETH W. SNYDER*, RICHARD R. RUSTANDI*[†], JOHN BIGGINS[‡], JAMES R. NORRIS*[†],
AND MARION C. THURNAUER*[§]

*Chemistry Division, Argonne National Laboratory, Argonne, IL 60439; [†]Department of Chemistry, The University of Chicago, Chicago, IL 60637; and [‡]Section of Biochemistry, Brown University, Providence, RI 02912

Communicated by Joseph J. Katz, August 2, 1991 (received for review May 16, 1991)

ABSTRACT The characteristic electron spin polarized electron paramagnetic resonance (ESP EPR) signal observed in photosystem I (PSI) has been previously assigned to a radical pair composed of the oxidized primary donor and a reduced vitamin K₁. Under conditions in which Bottin, H. & Sétif, P. [(1991), *Biochim. Biophys. Acta* 105, 331–336] proposed that A₁ is doubly reduced, the ESP EPR signal was not observed. Therefore, the ESP EPR signal can be directly attributed to A₁⁻, and vitamin K₁ can be assigned as this PSI acceptor. The ESP EPR signal was partially restored by removal of the chemical reductants.

Photosystem I (PSI) is responsible for photo-induced electron transfer from plastocyanin to ferredoxin. A number of outstanding questions remain regarding the generally accepted electron transfer pathway, P₇₀₀A₀A₁F_xF_aF_b of PSI, where P₇₀₀ is the primary chlorophyll donor; A₀ is a chlorophyll monomer; A₁ is the quinone vitamin K₁; and F_x, F_a, and F_b are iron-sulfur centers (1, 14). An electron paramagnetic resonance (EPR) signal has been observed that was attributed to the photoaccumulated A₁⁻ (2, 3). The properties of this signal were consistent with identifying A₁ with vitamin K₁ (2–4). However, the putative A₁⁻ EPR signal did not change when observed from PSI samples in which vitamin K₁ was either destroyed *in situ* by UV light (5) or was selectively substituted with deuterium (6). These conflicting results have prevented final assignment of the acceptor A₁ as vitamin K₁.

Recently, Sétif *et al.* proposed that in PSI preparations under highly reducing conditions [both photochemical (7) and chemical (8)] the singly reduced acceptor A₁⁻ is converted to a doubly reduced form, A₁²⁻. They suggested that A₁⁻ is reduced by back electron transfer from F_x⁻. In the unreduced sample, P₇₀₀⁺ decays with a half-time of 200–300 μs, arising from back electron transfer from either A₁⁻ or F_x⁻. In the reduced sample, P₇₀₀⁺ decays with a half-time of 40–50 ns, arising from back electron transfer from A₀⁻ (with electron transfer blocked from A₀⁻ to A₁²⁻). These results can be used to explain the discrepancies regarding the EPR observation of A₁⁻ under photoreducing conditions (7).

In related work, we demonstrated that the characteristic electron spin polarized EPR (ESP EPR) signal observed in PSI can be directly attributed to a radical pair composed of P₇₀₀⁺ and vitamin K₁⁻ (9). However, we did not address the identity of vitamin K₁ as the acceptor A₁. In the current work, we investigate the effect of double reduction of A₁ on the ESP EPR signal in PSI preparations.

EXPERIMENTAL PROCEDURES

D144 particles were isolated from commercial spinach by standard methods (10) and were solubilized in a pH 10.8 glycine buffer. The double reduction of A₁ was accomplished by the techniques of Sétif with sodium dithionite and methyl viologen (MV²⁺) (8). Only freshly prepared dithionite and MV²⁺ solutions were used. Anaerobic samples were allowed to incubate in the dark for ≈30 min before freezing. Unreduced samples were handled in the same fashion as the reduced samples, but without addition of dithionite and MV²⁺. Reduced samples were dialyzed against the glycine buffer to remove the chemical reductants and reoxidize the PSI acceptors (7). EPR and light-modulation EPR experiments were carried out on an X-band Varian instrument at cryogenic temperatures under conditions that have been described (9). Preliminary transient optical experiments, detecting P₇₀₀⁺ at 820 nm, were carried out in the laboratories of G. Closs (University of Chicago) and A. Trifunac (Argonne National Laboratory).

RESULTS AND DISCUSSION

In Fig. 1, we present the ESP EPR spectra for PSI samples that were unreduced and reduced and then dialyzed after reduction. Double reduction of A₁ eliminated the ESP EPR signal. Before irradiation the reduced sample showed the presence of reduced F_a and F_b, while the unreduced sample did not. From these EPR signals, we can confirm that the iron-sulfur centers were reduced in our treatments. In the preliminary transient optical experiments, chemical reduction caused an increase in the amplitude of the fast decay component relative to the slow decay component, confirming Sétif's results (7, 8). Dialysis of reduced samples against glycine buffer, followed by concentrating the sample to ≈40% of the optical density prior to dialysis, caused the ESP EPR signal to return to ≈50% of its original intensity (Fig. 1, trace c).

Under conditions in which A₁ was doubly reduced in PSI, the ESP EPR signal previously attributed to the radical pair P₇₀₀⁺ and vitamin K₁⁻ (9, 11) was destroyed. Therefore, the acceptor A₁ can be unequivocally assigned to vitamin K₁, and the ESP EPR signal observed in PSI can be directly attributed to a radical pair composed of P₇₀₀⁺ and A₁⁻. Considering the conflicting data surrounding the EPR observations of photoaccumulated A₁⁻ (5, 6, 12), the ESP EPR signal observed in PSI provides the best direct EPR probe of A₁⁻. A complete theoretical model (13) of this transient EPR signal may help determine whether A₁ is indeed the second electron acceptor in PSI and should provide structural information on the PSI

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ESP, electron spin polarization; EPR, electron paramagnetic resonance; PSI, photosystem I; MV²⁺, methyl viologen. [§]To whom reprint requests should be addressed.

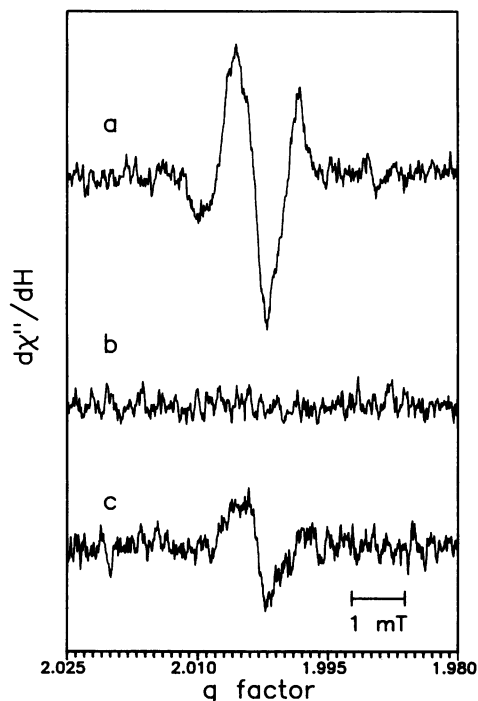


FIG. 1. ESP EPR signal of spinach PSI in pH 10.8 glycine buffer. Traces: a, untreated sample shows normal emission/absorption/emission pattern; b, sample reduced with 50 mM dithionite and 500 μM MV^{2+} followed by dark incubation; c, reduced sample from trace b dialyzed overnight against glycine buffer and then concentrated to 40% of original optical density. The ESP EPR signal returns to about half of the original intensity.

reaction center as it undergoes electron transfer. On a more general note, the parallels between PSI and the purple bacterial reaction center and photosystem II have been further elucidated. Quinones have roles as acceptors in all three photosynthetic systems.

Note Added in Proof. The altered shape of the ESP EPR signal in the dialyzed sample (Fig. 1, trace c) in comparison to the untreated sample (trace a) can be attributed to a perturbation of electron transfer kinetics by residual NV^{2+} . In a similarly prepared reduced/dialyzed sample, the ESP EPR signal was restored to the shape of the untreated sample by addition of 2 mM sodium ascorbate.

We thank Andong Liu (Argonne National Laboratory) and Daisy Zhang (University of Chicago) for assistance with the transient optical experiments. The work done at Argonne was supported by the U.S. Department of Energy, Office of Basic Energy Sciences, Division of Chemical Sciences under Contract W-31-109-Eng-38. The work done at Brown University was funded by the Competitive Research Grants Office of the United States Department of Agriculture 88-37130-4135.

1. Rutherford, A. W. & Heathcote, P. (1985) *Photosynth. Res.* **6**, 295–316.
2. Gast, P., Swarthoff, T., Ebskamp, F. C. R. & Hoff, A. J. (1983) *Biochim. Biophys. Acta* **722**, 163–175.
3. Bonnerjea, J. & Evans, M. C. W. (1982) *FEBS Lett.* **148**, 313–316.
4. Thurnauer, M. C. & Gast, P. (1985) *Photobiochem. Photobiophys.* **9**, 29–38.
5. Ziegler, K., Lockau, W. & Nitschke, W. (1987) *FEBS Lett.* **217**, 16–20.
6. Barry, B. A., Bender, C. J., McIntosh, L., Ferguson-Miller, S. & Babcock, G. T. (1988) *Isr. J. Chem.* **28**, 129–132.
7. Sétif, P. & Bottin, H. (1989) *Biochemistry* **28**, 2689–2697.
8. Bottin, H. & Sétif, P. (1991) *Biochim. Biophys. Acta* **1057**, 331–336.
9. Rustandi, R. R., Snyder, S. W., Feezel, L. L., Michalski, T. J., Norris, J. R., Thurnauer, M. C. & Biggins, J. (1990) *Biochemistry* **29**, 8030–8032.
10. Anderson, J. M. & Boardman, N. K. (1966) *Biochim. Biophys. Acta* **112**, 403–421.
11. Sieckmann, I., Van der Est, A., Bottin, H., Sétif, P. & Stehlik, D. (1991) *FEBS Lett.* **284**, 98–102.
12. Hauska, G. (1988) *Trends Biochem. Sci.* **13**, 415–416.
13. Norris, J. R., Morris, A. L., Thurnauer, M. C. & Tang, J. (1990) *J. Chem. Phys.* **92**, 4239–4249.
14. Golbeck, J. H. (1989) *Biochim. Biophys. Acta* **895**, 167–204.