DETECTION OF CHEMICALLY-INDUCED DYNAMIC ELECTRON POLARISATION (CIDEP) IN WHOLE CELLS AND MEMBRANE FRACTIONS OF *CHLOROBIUM LIMICOLA* f. *THIOSULPHATOPHILUM*

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Received 25 January 1982; revision received 8 March 1982

1. Introduction

Chemically-induced dynamic electron polarisation (CIDEP) in photosynthesis was first observed as strongly-polarised transient electron paramagnetic resonance (EPR) signals in spinach chloroplasts at room temperature [1]. These polarised signals (e.g., non-Boltzmann population of the electron spin sublevels) are proposed to arise from the photosystem I reaction centre via generation of a nascent radical pair during electron transfer from the excited singlet state of the primary electron donor P700 [2–4]. These polarisations (CIDEP) yield information about events associated with primary charge separation. CIDEP has not been observed in the photosystem II reaction centre of higher plants, but in reaction centre preparations and membrane fractions from purple photosynthetic bacteria after exposure to the detergent sodium dodecyl sulphate (SDS) [5,6]. In these preparations the primary electron acceptor ubiquinone and its associated high-spin ferrous ion are magnetically uncoupled.

Here, we report EPR transients exhibiting CIDEP in whole cells and membrane fractions of the green sulphur bacterium *C. l.* f. *thiosulphatophilum* at cryogenic temperatures. These signals do not arise from reaction centres analogous to those found in purple photosynthetic bacteria, as the preparations were not exposed to SDS, other detergent or denaturing agent. These results show that a reaction centre in green photosynthetic bacteria resembles photosystem I of cyanobacteria and higher plants.

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2. Materials and methods

Chlorobium limicola f. *thiosulphatophilum* (strain Tassjara) was grown as in [7]. The cells were harvested using a continuous action rotor, and the pellet stored at liquid nitrogen temperature. Freshly harvested cells were resuspended in a minimal volume of 0.01 M phosphate buffer (pH 7.4) containing 0.01 M sodium ascorbate sparged with oxygen-free nitrogen, and EPR samples prepared. Chlorosomes were prepared from frozen cells as in [7]. To prepare a partially-purified 'bacteriochlorophyll, reaction-centre complex' designated 'complex I' [8,9] we used a modification of the technique in [10].

EPR measurements were done using a Jeol FE-1X spectrometer equipped with an Oxford Instruments liquid helium cryostat. For kinetic measurements the response time of EPR spectrometer was improved to 30 μs, and the signals recorded on a Datalab DL920 transient recorder and Datalab 4000B signal averager. The excitation source was a Chromatix CMX-4 tunable flashlamp excited dye laser, set at 660 nm with a pulse length of $\sim$1 μs. All g-factors were measured against a standard, powdered, manganese oxide sample.

Aminoiminomethanesulfinic acid (AIMS) was purchased from Eastman Organic Chemicals (Eastman Kodak Co.). For experiments involving chemical reduction 18 mM AIMS was dissolved in 0.5 M glycine–KOH buffer (pH 10.9) containing 10 μM methyl viologen, and gassed with nitrogen until the methyl viologen was double reduced. Equal volumes of this solution and the sample to be reduced were mixed in an EPR tube and gassed with nitrogen before freezing in liquid nitrogen.

Published by Elsevier Biomedical Press
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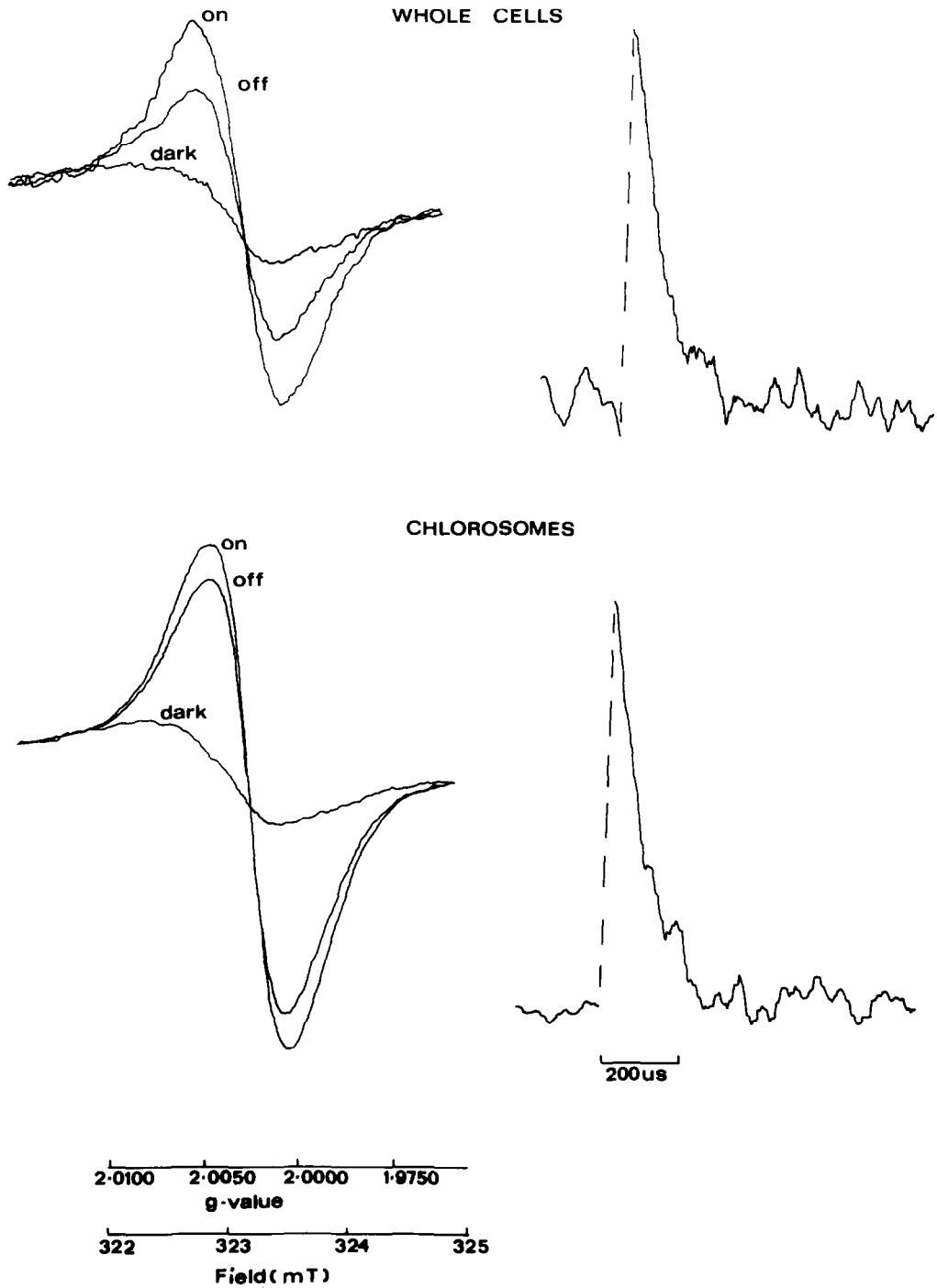

Fig.1. EPR signals in the $g = 2.00$ region induced by illumination of whole cells and chlorosomes of *C. l.* f. *thiosulphatophilum* at 50 K. Samples were prepared as in section 2 and frozen after 30 min in the dark. (Left) EPR spectra recorded before, during and after illumination of the sample in the cavity at 50 K. The instrument settings were: frequency, 9.0950 GHz; microwave power, 100 μW; modulation amplitude, 0.2 mT; scan rate, 2.5 mT/min; instrument gain, 1.25×10^3 (chlorosomes) or 2.5×10^3 (whole cells). The kinetic traces were recorded with the spectrometer set at $g = 2.0022$, and an instrument gain of 5×10^3, otherwise the instrument settings were unchanged. The signals were accumulated for 2048 scans (whole cells) and 1024 scans (chlorosomes).

3. Results

Fig.1 shows the EPR signals in the $g = 2.00$ region of the EPR spectrum of samples of whole cells and chlorosomes of *C. l.* f. *thiosulphatophilum* before, during and after illumination of cryogenic temperatures. In these samples, which were frozen in the dark in a buffer containing 0.01 M ascorbate, both an irreversible and reversible free radical were photo-induced. Both radicals were symmetrically centred at $g = 2.0025 \pm 0.0002$ with a peak-to-peak linewidth $\Delta H_{pp} = 9 \pm 0.2$ G as reported [9,11], and are attributed to photo-oxidation of P840, the primary electron donor thought to be a dimer of bacteriochlorophyll *a* molecules. Fig.1 also presents flash-induced transient EPR signals that are observed at $g = 2.0022$ in these samples. These signals decay with a $t_{1/2} \sim 100$ μs in the presence of a nominal microwave power of 100 μW and at 50 K. Using 'complex I' and pigment–protein complexes prepared from *Prosthecochloris aestuarii* [11] the reversible P840 decayed with $t_{1/2} = 13$ ms at 5 K, and a triplet EPR signal photo-induced in the same samples decayed with av. $t_{1/2} = 290 \pm 30$ μs at 5 K. The flash-induced transients exhibited in fig.1 decay faster than either reversible P840 or the triplet, suggesting that they arise from another species, so a point-by-point kinetic spectrum of these flash-induced transients was obtained (fig.2). To maximise the signal-to-noise ratio a partially-purified 'bacteriochlorophyll reaction-centre complex' 'complex I' [8,9] was used, which is analogous to a chromatophore from purple photosynthetic bacteria, as it contains 80–100 bacteriochlorophylls/reaction centre. Fig.2 shows the EPR signals at $g = 200$ induced in this sample by illumination at 50 K, again P840 is irreversibly and reversibly photo-induced. The point-by-point spectrum presented in fig.2 was compiled by measuring the height of the transient signal 30 μs after the flash. This spectrum is considerably narrower than the peak-to-peak linewidth of P840, and is asymmetric, with a negative lobe on the high-field side and the low-field side. Representative kinetic traces obtained at the points in the kinetic spectrum indicated are shown in fig.3, and all have the same decay kinetics. This demonstrates that the kinetic spectrum is that of a single species, and is the result of the superimposition of both emissive and enhanced absorption signals.

These observations are all characteristic of CIDEP [2–5]. The decay of spin-polarised transients, which reflects a return to a Boltzmann population, is depen-

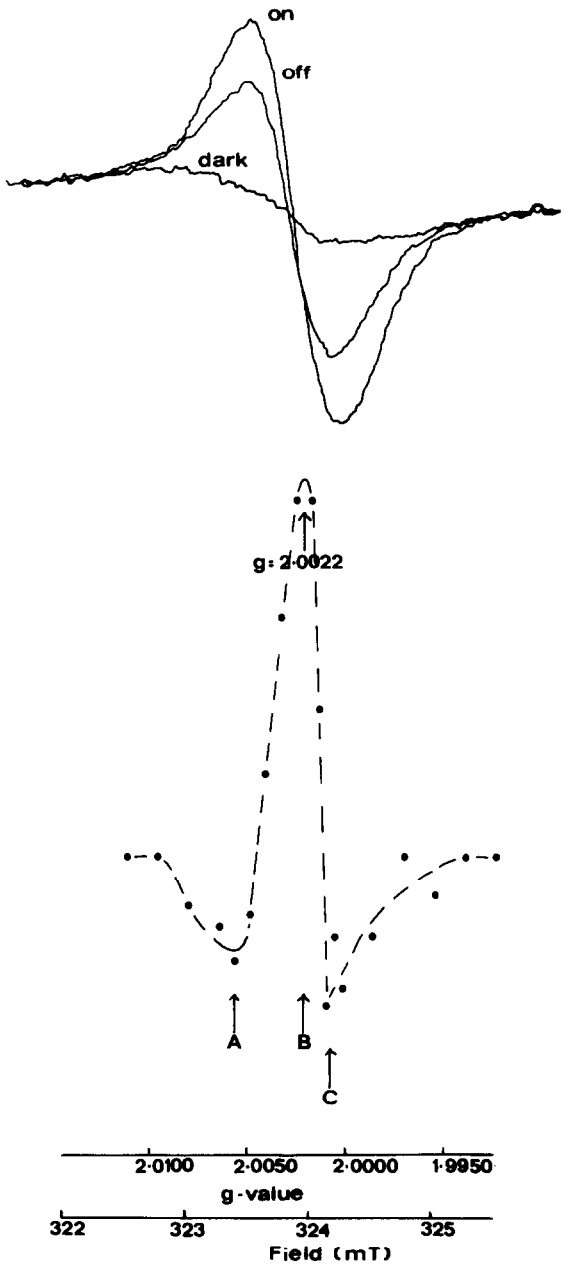

Fig.2. EPR spectra and a kinetic point-by-point spectrum observed in 'complex I' prepared from *C. l.* f. *thiosulphatophilum*. The sample was prepared as in section 2 and frozen after 30 min in the dark. The EPR spectra were recorded before, during and after illumination in the cavity at 50 K. The instrument settings were: frequency, 9.0975 GHz; microwave power, 50 μW; modulation amplitude, 0.2 mT; scan rate, 2.5×10^3. The point-by-point spectrum was compiled by measuring the response 30 μs after the flash at an instrument gain of 5×10^3, the signals were accumulated for 1024 scans.

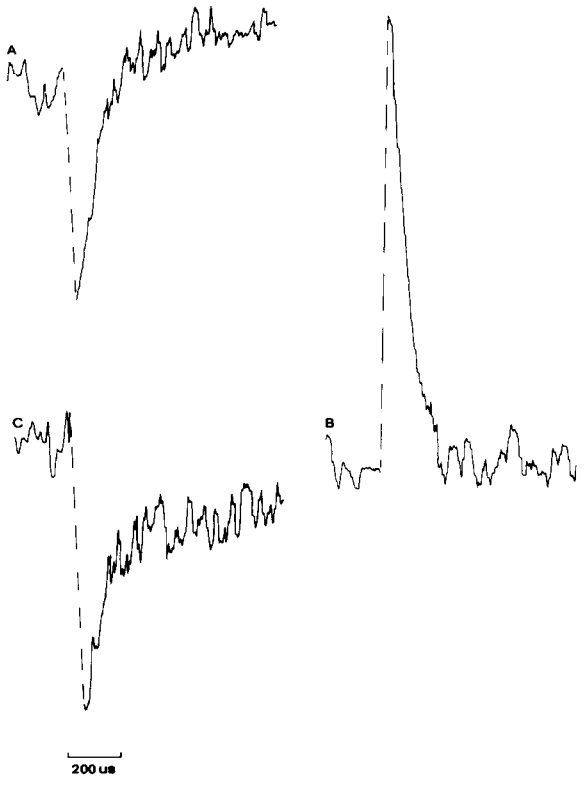

Fig.3. Kinetics of the flash-induced EPR signal in 'complex I' prepared from *C. l.* f. *thiosulphatophilum* at 50 K. These representative traces were recorded as detailed in fig.2, and at the positions indicated in fig.2; (A,C) recorded at an instrument gain of 5×10^3; (B) at a gain of 2.5×10^3.

dent on microwave power. (The $1/e$ time required to establish a Boltzmann population is termed t_1.) So care was taken to ensure that the CIDEP signals were being matured at a non-saturating microwave power, by monitoring the effect of microwave power on the rate of decay of the flash-induced transients. The t_1 effective was plotted against microwave power, and extrapolating to zero microwave power yielded a t_1 of 230 ± 30 μs.

We measured the redox potential span of the radical pair giving rise to CIDEP: the signals were abolished when 'complex I' was incubated with 0.04 M potassium ferricyanide for 10 min in the dark before freezing the EPR sample (>+400 mV). The CIDEP signals were also abolished when 'complex I' was incubated in the dark at pH 10.9 with 9 mM AIMS for 30 min (<–600 mV) and the sample illuminated for 60 s and frozen under illumination.

4. Discussion

Purple photosynthetic bacteria contain one photosynthetic reaction centre capable of reducing a primary electron acceptor of redox potential mid-point (E_m) –100 to –200 mV. The reduction of pyridine nucleotides (NAD^+, E_m = 340 mV) and ferredoxin is achieved by reverse electron flow at the expense of ATP provided by cyclic electron transport. In contrast the photosynthetic membranes of cyanobacteria and higher plants contain photosystem I which reduces a membrane-bound iron–sulphur centre (centre A, E_m = 550 mV) which can directly reduce ferredoxin and $NADP^+$. Similarly membrane fractions of the green sulphur bacterium *C. l.* f. *thiosulphatophilum* are capable of reducing NAD^+ via ferredoxin in the light in the presence of uncouplers [12–14]. This observation suggests that *C. l.* f. *thiosulphatophilum* contains a photosynthetic reaction centre that resembles photosystem I. Several reports have suggested that the acceptor side of a reaction centre in green photosynthetic bacteria contains a low potential iron–sulphur centre capable of reducing NAD^+ and ferredoxin directly [7,15,16], but the reports differ as to the EPR characteristics of this acceptor.

In the chlorosomes and 'complex I' isolated from *C. l.* f. *thiosulphatophilum* we have observed that the irreversible photo-oxidation of P840 is accompanied by the irreversible photoreduction of the component in [7]. In these preparations no other signals are photo-induced at cryogenic temperatures, except reversible P840. The observation of CIDEP in these preparations clearly demonstrates that they contain a reaction centre quite different from those found in purple photosynthetic bacteria and similar to photosystem I. The lineshape of the kinetic CIDEP spectrum resembles that obtained from photosystem I reaction centres [17,18], but not the spectrum obtained from the reaction centres of purple photosynthetic bacteria after exposure to SDS [5]. In addition, the signals obtained from purple photosynthetic bacteria [5] showed a complex time- and field-dependency, with the decay kinetics altering from point to point in the spectrum, and the line-shape altering depending on how long after the flash the height of the signal was measured. Presumably the acceptor side of the reaction centre in green photosynthetic bacteria resembles photosystem I, and our results suggest that a primary electron acceptor analogous to centre A is the $g = 1.90$ iron–sulphur centre photoreduced at cryogenic temperatures [7].

Acknowledgements

This work was supported by grants from the Science and Engineering Research Council, The Royal Society, and the Central Research Fund. We thank Dr M. C. W. Evans for advice and encouragement, Miss M. Ayres and Miss L. Richardson for technical assistance. J. T. W. acknowledges the hospitality of M. C. W. E. and partial financial support for this work.

References

[1] Blankenship, R., McGuire, A. and Sauer, K. (1975) Proc. Natl. Acad. Sci. USA 72, 4943–4947.
[2] Dismukes, G. C., McGuire, A., Blankenship, R. and Sauer, K. (1978) Biophys. J. 21, 239–256.
[3] Friesner, R., Dismukes, G. C. and Sauer, K. (1979) Biophys. J. 25, 277–294.
[4] Warden, J. T. and Adrianowycz, O. A. (1982) in: Proc. 5th Intl. Congr. Photosynth. Greece, 1981 (Akonoglou, G. ed) in press.
[5] Hoff, A. J., Gast, P. and Romijn, J. C. (1977) FEBS Lett. 73, 185–190.
[6] Gast, P. and Hoff, A. J. (1979) Biochim. Biophys. Acta 548, 520–535.
[7] Jennings, J. V. and Evans, M. C. W. (1977) FEBS Lett. 75, 33–36.
[8] Olson, J. M., Philipson, K. D. and Sauer, K. (1973) Biochim. Biophys. Acta 292, 206–217.
[9] Olson, J. M., Prince, R. C. and Brune, D. C. (1977) Brookhaven Symp. Biol. 28, 238–245.
[10] Fowler, C. F., Nugent, N. A. and Fuller, R. C. (1971) Proc. Natl. Acad. Sci. USA 68, 2278–2282.
[11] Swarthoff, T., Gast, P. and Hoff, A. J. (1981) FEBS Lett. 127, 83–86.
[12] Evans, M. C. W. (1969) Prog. Photosynth. Res. 3, 1474–1475.
[13] Buchanan, B. B. and Evans, M. C. W. (1969) Biochim. Biophys. Acta 180, 123–129.
[14] Knaff, D. B. (1978) in: The Photosynthetic Bacteria (Clayton, R. K. and Sistrom, W. R. eds) pp. 629–640, Plenum, New York.
[15] Knaff, D. B. and Malkin, R. (1976) Biochim. Biophys. Acta 430, 244–252.
[16] Swarthoff, T., Gast, P., Hoff, A. J. and Amesz, J. (1981) FEBS Lett. 130, 93–98.
[17] McIntosh, A. R. and Bolton, J. R. (1976) Nature 263, 443–445.
[18] McIntosh, A. R., Manikowski, H., Wong, S. K., Taylor, C. P. S. and Bolton, J. R. (1979) Biochem. Biophys. Res. Commun. 87, 605–612.