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Characterisation of electron acceptors A_0 and A_1 in cyanobacterial Photosystem I

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Preparations of cyanobacterial Photosystem I were compared to those of pea Photosystem I using ESR spectroscopy. Photoreduced samples were illuminated at cryogenic temperatures with ESR spectra taken at 205 K. After illumination at 205 K and 230 K, signals appeared which correspond to the shape and position of the signals assigned to A_1 and A_0, respectively, in higher plant Photosystem I.

Photosystem I catalyses the light-induced transfer of electrons from the primary electron donor (P-700) along a chain of components of soluble ferredoxin. In higher plants these components consist of the secondary electron acceptors, iron-sulphur centres *X*, A and B (identified by ESR, optical and Mossbauer spectroscopy [1]) and the intermediary acceptors, A_0 (probably a chlorophyll *a*) and A_1 (which is probably a quinone) both identified by optical and ESR spectroscopy [2,3]. A simple scheme for the transfer of electrons from P-700 to soluble ferredoxin might be (Ref. 4):

$$\text{P-700}-A_0-A_1-FeS_X-FeS_B-FeS_A-\text{Fd}$$

The iron-sulphur centres have also been identified in cyanobacteria [5].

Here we provide evidence, using ESR spectroscopy, that cyanobacterial Photosystem I also contains the acceptors A_0 and A_1.

Photosystem I particles were prepared from *Synechococcus leopoliensis (Anacystis nidulans* UTEX 625), *Phormidium laminosum*, and from pea (*Pisum sativum* var. Feltham first) leaves. Photosystem I from *S. leopoliensis* was prepared using sonicated cells with a 1.5% digitonin digestion followed by a 1.5% Triton X-100 digestion using a method based on that in Ref. 6; the sepharose column purification stage was omitted, and replaced with a more effective hydroxyapatite column. *P. laminosum* Photosystem I particles were prepared from membranes solubilised in lauryldimethylamine *N*-oxide (LDAO) as in Ref. 7. The pellet formed after LDAO extraction was solubilised in Triton X-100. This was centrifuged and the supernatant (containing the Photosystem I) retained. Pea Photosystem I particles were prepared from the supernatant following the Triton X-100 digestion of magnesium-stacked chloroplasts [8]. All Photosystem I particles were finally purified on a hydroxyapatite column as in Ref. 9 with *P. laminosum* Photosystem I particles requir-

Abbreviations: Chl, chlorophyll; ESR, electron spin resonance; LDAO, lauryldimethylamine *N*-oxide.

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ing two passes down the column. The final P-700/Chl ratios were 1 : 56, 1 : 90 and 1 : 45 for *S. leopoliensis, P. laminosum* and pea Photosystem I, respectively.

For ESR spectroscopy, anaerobic Photosystem I particles were photoreduced in the presence of dithionite in ESR tubes, prior to freezing with liquid nitrogen (this treatment reduces the iron-sulphur centres leaving A_0 and A_1 oxidised [10]). ESR measurements were carried out at 205 K. Illuminations to photoreduce A_1 were carried out at 205 K, and at 230 K for A_0, as described in Ref. 10.

Illumination of Photosystem I at temperatures around 200 K has been found to result in the photoaccumulation of reduced electron acceptors.

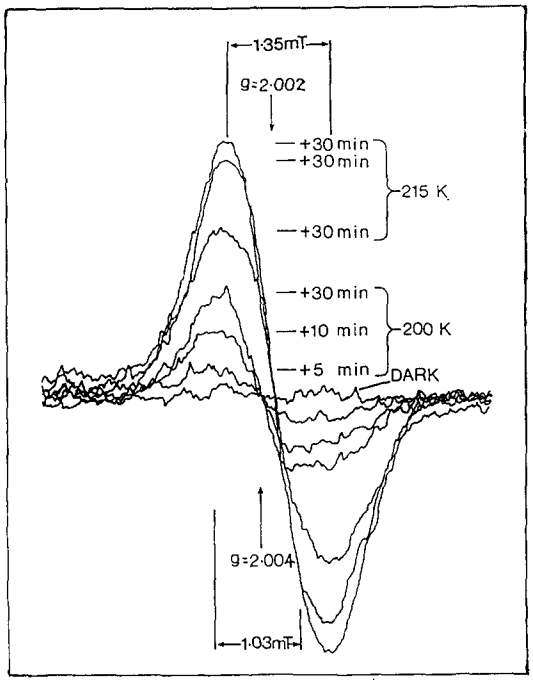

Fig. 1. ESR spectra of A_1 and A_0 after illumination (for stated time increments) of pea Photosystem I at 200 K (A_1) and 215 K (A_1 and A_0). Measurements were made using a Jeol FE-1X spectrometer with 100 kHz field modulation. The temperature of the Oxford Instruments cryostat (containing the ESR tube holding the Photosystem I sample) was measured using a carbon thermometer during assays; and *g*-values measured using a powdered manganese oxide sample as a standard. Illumination during preparation and measurement was provided by a Barr & Stroud 150 W fibre optic light source. Instrument settings were: microwave power, 100 W; frequency, 9.043 GHz; modulation amplitude, 0.2 mT.

Photoaccumulation of reduced electron acceptors was carried out under identical conditions for pea, *S. leopoliensis* and *P. laminosum* Photosystem I preparations. Fig. 1 shows an ESR spectrum, in the $g = 2.00$ region, of pea Photosystem I. The progressive appearence of the ESR signals attributed to A_1 alone, and A_0 and A_1 combined, can be seen upon illumination [10]. Illumination at 200–205 K causes the appearance, in pea photosystem I, of the asymmetric signal 1.03 mT wide with $g = 2.004$ which is characteristic of A_1. If the temperature during illumination was then raised to 215–230 K, the ESR signal broadened to 1.35 mT and was centred at $g = 2.0025$, this being characteristic of the combined signals of A_0 and A_1 [2,3,10]. The appearance of the $g = 2.00$ signal has been shown to be temperature sensitive, being more rapid at 230 K than 205 K [11].

Figs. 2 and 3 show the ESR spectra of *S. leopoliensis* and *P. laminosum* Photosystem I, respectively. Consecutive illuminations at 205 K caused the appearance of signals 1.03 mT wide centred at $g = 2.005$ in *S. leopoliensis* and 1.08 mT wide, centred at $g = 2.005$ in *P. laminosum*. It can be seen that these values are very similar to A_1

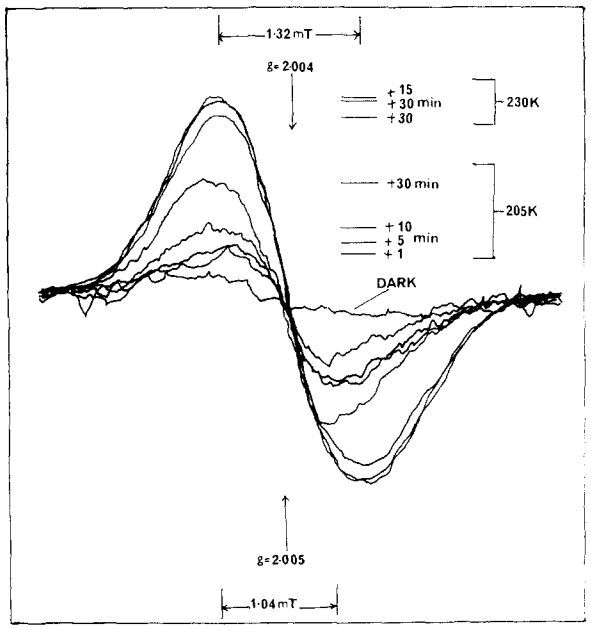

Fig. 2. ESR spectra of A_1 and A_0 after illumination of *S. leopoliensis* Photosystem I at 205 K and 230 K. The spectra were recorded at 205 K with microwave frequency, 9.033 GHz.

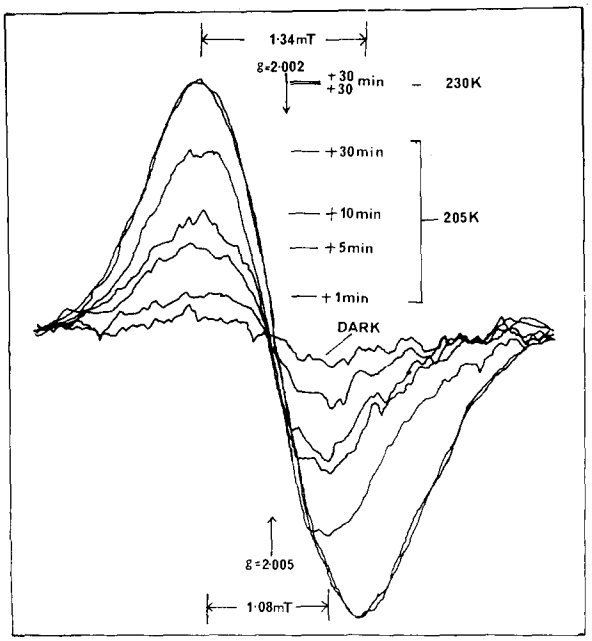

Fig. 3. ESR spectra of A_1 and A_0 after illumination of *P. laminosum* Photosystem I at 205 K and 230 K. The spectra were recorded at 205 K with microwave frequency, 9.051 GHz.

signals in pea and spinach (Table I). Subsequent illumination at 230 K caused the broadening of these signals to 1.32 mT in *S. leopoliensis* and 1.34 mT in *P. laminosum* with a shift in *g*-values to 2.004 and 2.002, respectively. These values correspond to the combined signals of A_0 and A_1 in pea and spinach (Table I).

This provides strong evidence for the existence of A_0 and A_1 in cyanobacterial Photosystem I. It seems possible that A_1 in plant Photosystem I is phylloquinone; it has already been suggested that A_1 is a quinone [10,12] and phylloquinone copurifies with P-700 in a ratio of 2 : 1 in green plants [13]. The position and shape of the ESR signals from cyanobacterial Photosystem I presented in this paper suggest that A_1 is phylloquinone in cyanobacteria too. A_0 has been suggested as a Chl *a* monomer (identified by ESR and optical studies [2,3,10]. Optical difference spectra have been obtained by picosecond spectroscopy and photoaccumulation techniques under conditions where changes were expected due to reduction of A_0. However, there is discrepancy between the findings. Absorption minima at 693 nm [14] and 670 nm [10] upon A_0 reduction have been reported. Further work is required to obtain the definitive difference spectrum for A_0/A_0^-.

TABLE I

COMPARISON OF LINE-WIDTHS AND *g*-VALUES OF ESR SIGNALS PHOTOACCUMULATED AT 205 K (A_1) AND 230 K (A_0) IN HIGHER PLANT AND CYANOBACTERIAL PHOTOSYSTEM I

	Temperature (K)	Signal width (mT)	*g*-Value
Pea	205	1.03	2.0040
	230	1.35	2.0025
Spinach	205	1.05	2.0050
	230	1.34	2.0030
Synechococcus	205	1.03	2.0050
	230	1.32	2.0040
Phormidium	205	1.08	2.0050
	230	1.34	2.0020

The results presented here, together with the earlier identification of the iron-sulphur centres *X*, A, and B in cyanobacterial Photosystem I complexes, show a close similarity between cyanobacterial and higher plant Photosystem I. Hence, this cyanobacterial system can be used as a model for higher plants. Analysis of the comparatively simple prokaryotic genome of cyanobacteria, with respect to Photosystem I, should therefore provide structural information of direct relevance to higher plants.

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