EPR SPECTRA OF PHOTOSYSTEM I CONSTITUENTS IN HETEROCYST PREPARATIONS FROM *ANABAENA CYLINDRICA*

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1. Introduction

Heterocysts are specialized cells formed by differentiation of vegetative cells, in filamentous nitrogen-fixing blue-green algae [1]. They differ from the vegetative cells in their pigment composition [2], and lack photosystem II-mediated activities [3]. However they contain *P*-700 and a *c*-type cytochrome [3] and have recently been found to carry out various photochemical reactions associated with Photosystem I [4,5].

It is possible to separate and purify intact heterocysts, which retain nitrogenase activity and a modified photosynthetic apparatus. In this paper we describe investigation of isolated heterocyst preparations from *Anabaena cylindrica*, using electron paramagnetic resonance (EPR) spectroscopy, which demonstrate the existence of Photosystem I components *P*-700 and bound iron–sulphur proteins, and provide indirect evidence for the presence of a third electron acceptor. Quantitative comparison with vegetative cells indicates that heterocysts are enriched in Photosystem I components.

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2. Materials and methods

Growth of N_2-fixing *Anabaena* cells, and separation of heterocysts and vegetative cells were as previously described by Tel-Or and Stewart [5].

Samples were used both whole, and after disruption with the French Press at a pressure of 16 000 pounds/inch^2. For comparison of heterocysts and vegetative cells, all samples were diluted with 30 mM sodium *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonate (HEPES) + 1 mM EDTA, pH 7.5 to a final concentration of 10 mg chlorophyll/ml.

EPR samples were prepared in duplicate, in tubes matched for internal diameter (about 3 mm). Samples were illuminated during freezing, where required, with a 300 W projector. EPR measurements were performed on a Varian E4 spectrometer, using a flow of cold helium gas to cool the sample.

3. Results and discussion

Samples of heterocysts in the oxidized state as prepared, showed an EPR signal, around $g = 2.00$, typical of Cu^{2+} (fig.1(a)). Since many copper proteins have $g_\perp$ in this position however, it is difficult to identify this component unambiguously. Plastocyanin has been detected by EPR in whole cells of *Anacystis nidulans*, [6], so it is probable that this represents

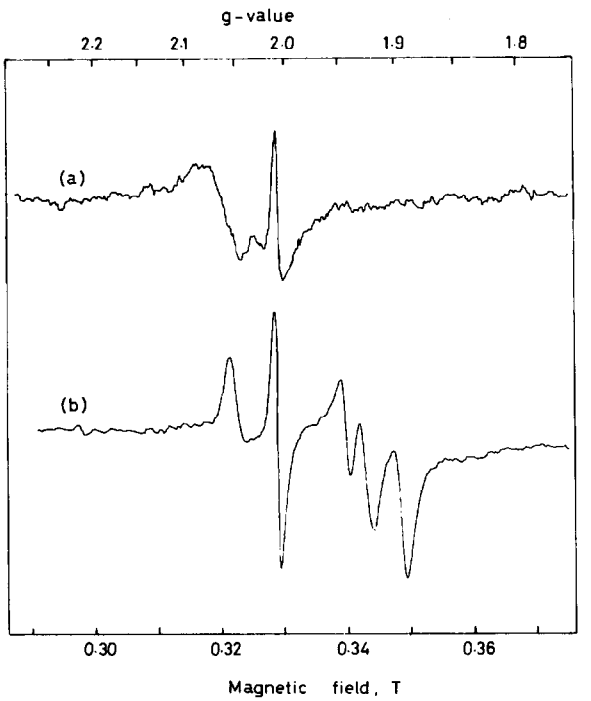

Fig.1. EPR spectra of heterocysts prepared as described in the Materials and methods section, without French press treatment. (a) As prepared, (b) treated with 5 mM dithionite and illuminated for 2 min before freezing. Spectra were recorded at 16°K, with the following instrument settings: microwave power, 20 mW, frequency, 9.2. GHz, modulation amplitude, 10 G, frequency 100 KHz.

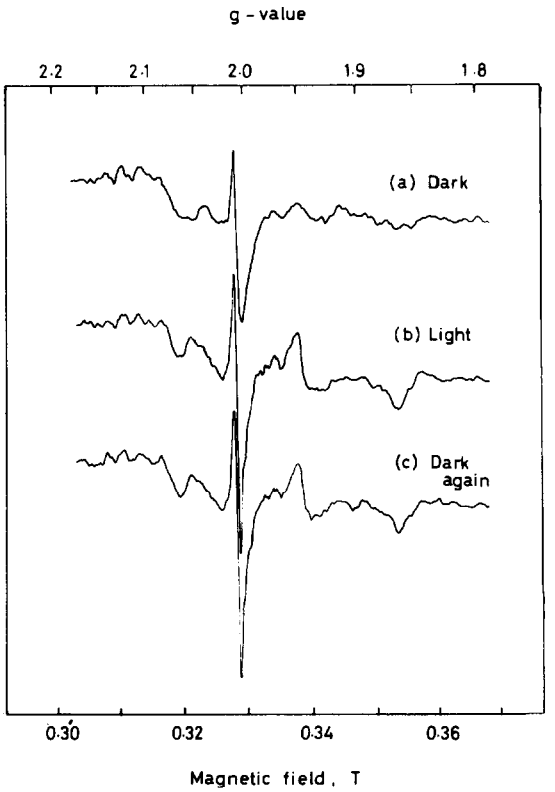

Fig.2. Spectra of heterocysts treated with the French Press, treated with 10 mM ascorbate + 0.1 mM DCIP and kept in the dark for 2 min before freezing. (a) As prepared, in the dark, (b) illuminated with a 1000 W projector, (c) dark again. Conditions of measurement were as for fig.1, except that the temperature was 20°K.

at least a proportion of the signal. However cytochrome oxidase which also gives a signal in this region [7] may also be present in observable amounts. Moreover, the $g = 2.05$ signal was not completely removed by reduction with ascorbate and 2,6-dichlorophenolindophenol (DCIP) (cf. fig.2a) so that another, possibly non-protein, form of copper may have been present in the heterocyst preparations. Other features of the spectrum of the oxidized preparations are a free radical at $g = 2.003$, presumably due to chlorophyll radicals. In the spectra at low temperatures (fig.1) this signal was highly saturated. There was also a signal at about $g = 2.02$, seen as a small peak in fig.1(a). This was only seen at low temperatures (below 30°K) and the variation in size of the signal in different samples indicated that it was independent of the Cu^{2+} signal. This signal may represent a respiratory chain component like the high-potential iron–sulphur protein (HiPIP)-type components with narrow signals at $g = 2.02$ in the oxidized state that have been detected in the mitochondrial respiratory chain [8].

The heterocyst preparations were reduced by treatment with dithionite and illumination before freezing (fig.1(b)). The signals assigned to Cu^{2+} and the HiPIP-type signal disappeared, and the spectrum at $g = 2.05$, 1.94, 1.92 and 1.89 appeared, typical of the membrane-bound iron–sulphur proteins of the primary acceptor complex of Photosystem I [9–11]. This signal has been interpreted in terms of two iron–sulphur centres, A and B [12].

Figure 2 shows the effect of illumination at 20°K, of a heterocyst sample prepared in the dark. The size of the free radical increased on illumination and a

signal at $g = 2.05$, 1.94 and 1.86 appeared, and remained when the light was turned off. This behaviour has previously been observed with lamellar preparations from *A. cylindrica* [13] and indicates the photo-oxidation of *P*-700 and reduction of the bound iron–sulphur centre A of the Photosystem I complex [14]. As with chloroplasts, the signal at $g = 1.86$ disappears on complete reduction, possibly becoming hidden under the signal at $g = 1.89$ [12].

When iron–sulphur centres A and B of Photosystem I are reduced before freezing the sample, illumination at 20°K results in photo-oxidation of *P*-700 which is reversible and therefore not linked to reduction of A and B. Although there is controversy about the origin of this reversible signal [15] there is mounting evidence it is linked to the reversible reduction of a third electron-accepting species (termed X) [11,16]. In concentrated samples of purified Photosystem I particles it is possible to detect a very broad signal due to this component, at $g = 1.76$, 1.88 and 2.08 in preparations from chloroplasts [17, 18] and the blue-green alga *Chlorogloea fritschii* [11]. The sample not sufficiently concentrated to observe this in the present experiments, but it was possible to detect the reversible photo-oxidation of a radical species, presumably *P*-700, in samples that were reduced with dithionite and illuminated during freezing, conditions that lead to complete reduction of centres A and B [9]. These samples still showed a radical signal at $g = 2.00$ in the dark, presumably due to chlorophyll (fig.1(b)). This radical gradually became smaller if the period of incubation with dithionite before freezing was prolonged. We therefore chose standard conditions for preparing the samples, illuminating them in the presence of 5 mM dithionite at 20°C for 2 min before freezing. On illuminating these samples at 20°K an increase in the radical signal was observed, which reversed when the light was turned off; in broken heterocyst preparations the extent was about 30% of the total radical signal. Although the extent of reversible photo-oxidation was variable, depending on the conditions of reduction of the samples, it was consistently higher for the heterocyst preparations, both broken and unbroken, than for the equivalent filament preparation (table 1).

Table 1 shows a comparison of the sizes of the EPR signals of the Photosystem I components, in preparations of heterocysts and vegetative filaments of the same chlorophyll concentration. There appears to be significantly more of these components in the heterocysts than in the vegetative cells. These results suggest that synthesis of these components takes place during the process of differentiation of a vegetative cell to a mature heterocyst. Photosytem I appears to be essential for the metabolic activity of the heterocysts, and it is possible that its function is to provide ATP and reduced ferredoxin for nitrogen fixation.

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Table 1
The relative amounts of bound iron–sulphur proteins and reversible *P*-700 signal in heterocysts and vegetative cells of *Anabaena cylindrica*

	Heterocysts	Vegetative cells
Chlorophyll concentration (mg/ml)	10	10
Protein concentration (mg/ml)	8.2	10.6
Bound iron–sulphur proteins (arbitary units)	23	16
Reversible *P*-700 signal (arbitary units)	19	12

Cells were prepared [5] and broken by French Press treatment. EPR spectra were recorded as for fig.1.

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