THE EFFECT OF THE REDOX STATE OF THE BOUND IRON-SULPHUR CENTRES IN SPINACH CHLOROPLASTS ON THE REVERSIBILITY OF P700 PHOTOOXIDATION AT LOW TEMPERATURES.

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Photooxidation of P700 at cryogenic temperatures is coupled to the photoreduction of two membrane-bound iron-sulphur centres. This process is irreversible over short time periods at 20K. We have now shown that if the iron-sulphur centres are chemically reduced before freezing, P700 photooxidation is no longer coupled to reduction of the iron-sulphur centres. This photooxidation is completely reversible. We therefore conclude that the iron-sulphur centres are secondary electron acceptors and that an unknown primary electron acceptor transfers electrons from P700 to the iron-sulphur centres.

INTRODUCTION

The primary photochemical reaction in Photosystem I (PSI) is thought to be the photooxidation of the reaction centre chlorophyll P700. At cryogenic temperature this photooxidation has usually been thought to be irreversible[1]. Malkin & Bearden[2] found that chloroplasts contain membrane-bound iron-sulphur proteins, and showed that these proteins were photoreduced at cryogenic temperatures in parallel with the photooxidation of P700. These results, together with experiments showing the concentration of the iron-sulphur centres in PSI particles[3,4], and the extremely low redox potentials of the centres[5,6], suggested that they might be the primary electron acceptors of PSI. However Mayne & Rubinstein[7] presented some evidence that P700 photooxidation might be partially reversible at low temperature. Bolton et al[8] have recently shown that in Triton PSI particles P700 photooxidation at low temperatures was partially reversible. The extent of the reversible reaction varied

between preparations, 10-30% of the P700 photooxidation being reversible. They did not observe any reversible reduction of the iron-sulphur centres. We have confirmed these results using French press particles[9]. Bolton suggested that, as the photooxidation of P700 was partially reversible, while the photooreduction of the iron-sulphur centres was apparently irreversible, an unknown component must function as the primary electron acceptor of PSI. They have recently presented evidence for the existence of an EPR-detectable component which may be the primary acceptor[8]. Our preliminary experiments supported these conclusions[9]. Visser et al[10] also observed the reversible photooxidation of a small part of the P700 in their preparations, but reported that the photoreduction of the iron-sulphur protein was also reversible.

We have now investigated the effect of the redox state of the bound iron-sulphur centres on the extent to which the photooxidation of P700 is reversible at low temperatures.

MATERIALS AND METHODS

PSI particles were prepared from spinach as described previously using the French press[4]. The particles were suspended in 0.1M Tris: HCl pH 8.0 and stored frozen in liquid nitrogen. The chlorophyll concentration in all samples used was 3.0 mg/ml. EPR samples were prepared in silica tubes (3mm internal diameter) under an atmosphere of nitrogen. The reduction of the particles by dithionite at pH 9.0 over long periods of time (30-60 mins.) was done in an anaerobic vessel and samples were transferred to gassed EPR tubes through a stainless steel tube[6]. Samples were poised at measured oxidation reduction potentials as described previously[6]. EPR spectroscopy was done with a Varian E4 spectrometer using a helium flow system to cool the sample. Samples were illuminated in the spectrometer cavity with a 250 watt projector through a 2 cm water filter. The light intensity at the sample was approximately 2 x 10^4 ergs/cm^2/sec. At maximum intensity, the illumination was found to increase the sample temperature by less than 0.25°K.

RESULTS AND DISCUSSION

If PSI particles in the absence of any added reducing agent are illuminated at low temperature (below 30 K) the P700, as observed by the change in the

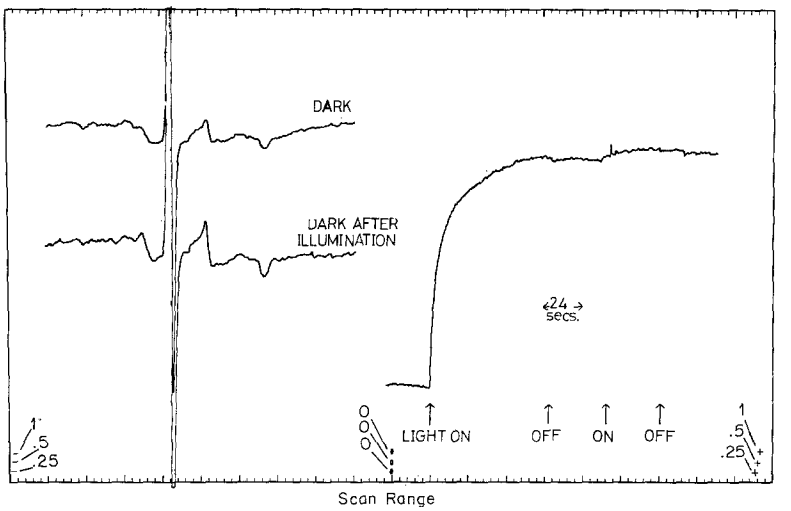

FIG.1 The effect of illumination on the EPR signal of P700 in PSI particles. LEFT. The EPR spectrum in the g = 2.00 region Instrument settings Frequency 9.26 GHz. Power 20 mW. Modulation Amplitude 109. Scan Rate 500 gauss/min. Gain 1500. RIGHT. Time course of the effect of illumination on the P700 signal. Instrument settings as above except that the change in amplitude of the g = 2.00 signal was followed with time.

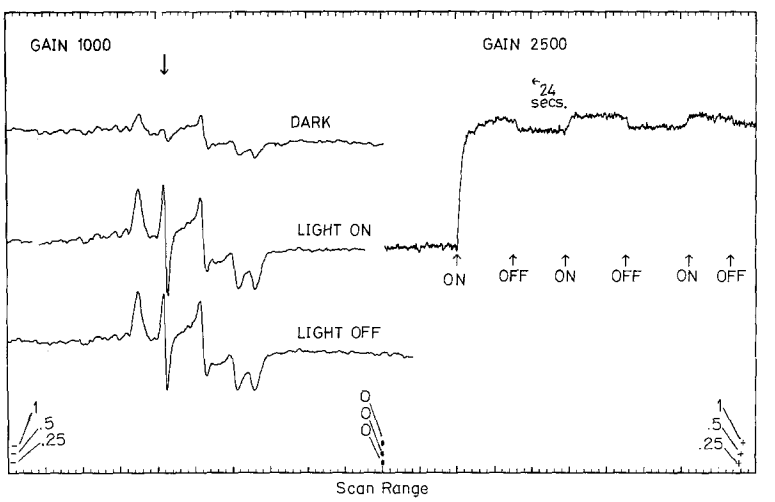

FIG.2 The effect of illumination on the EPR spectrum of PSI particles frozen after short exposure to sodium dithionite. LEFT. The EPR spectrum in the g = 2.00 region. RIGHT. Time course of the effect of illumination on the P700 signal. Instrument settings as in Fig.1 with gain settings as indicated.

EPR signal at g = 2.00, becomes oxidized (Fig.1). The oxidation under the conditions used is biphasic, showing an initial fast reaction followed by a slow phase. The photoreduction of the iron-sulphur proteins shown by signals at g = 1.86, 1.94 & 2.05 shows the same kinetics. The proportions of the fast and slow phases depend on the light intensity. The slow phase represents the time taken for sufficient light to penetrate the sample, and is not seen in dilute samples for which the light intensity is saturating. In the absence of any reducing agent the photooxidation of P700 is completely irreversible.

If the PSI particles are frozen after only short exposure (1 min.) in the dark to dithionite, little or none of the iron-sulphur centres are in the reduced state. When these frozen samples are illuminated, the kinetics of photoreduction of the iron-sulphur centres and photooxidation of P700 are similar to those in the absence of dithionite (Fig.2). However a small part of the P700 photooxidation is reversible. We have been unable to observe any parallel reversibility in the photoreduction of the iron-sulphur proteins. We cannot therefore support the observations of Visser et al[10] on this reaction.

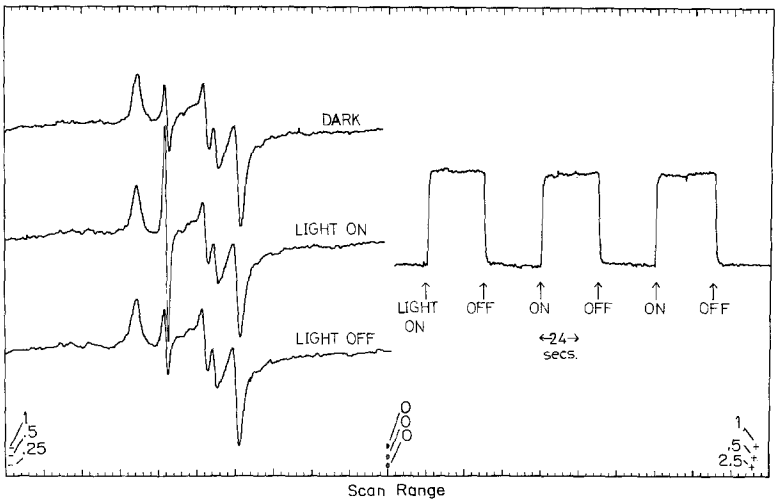

FIG.3 The effect of illumination on the EPR spectrum of PSI particles fully reduced by sodium dithionite. LEFT. The EPR spectrum in the g = 2.00 region. RIGHT. Time course of the effect of illumination on the P700 signal Instrument settings as in Fig.1. Gain 1000.

If PSI particles which have been frozen and thawed are exposed to sodium dithionite for long periods (30-60 min.) at pH 9.0, the iron-sulphur centres are extensively reduced in the dark. The extent of reduction varies with time and preparation. The experiments described here were done with samples in which 90-100% reduction had occurred after 45-60 min. exposure to dithionite. Fig.3 shows that in such samples illumination results in the photooxidation of P700 with the appearance of the characteristic g = 2.00 signal. When the light is turned off the signal disappears again. (Even under these conditions a small part (5-10%) of the P700 signal may be irreversibly "frozen in"). The rise of the P700 signal is too fast for our recording equipment and corresponds to 70-80% of the irreversible P700 signal in similar samples without dithionite. The size of the P700 signal depends on light intensity in the range which we have used. It seems likely that if a high enough light intensity could be used the reversible signal would be 100% of the irreversible signal observed in the absence of dithionite. There is no change in the redox state of the iron-sulphur protein corresponding to the reversible photooxidation of the P700. We therefore conclude, in agreement with the findings

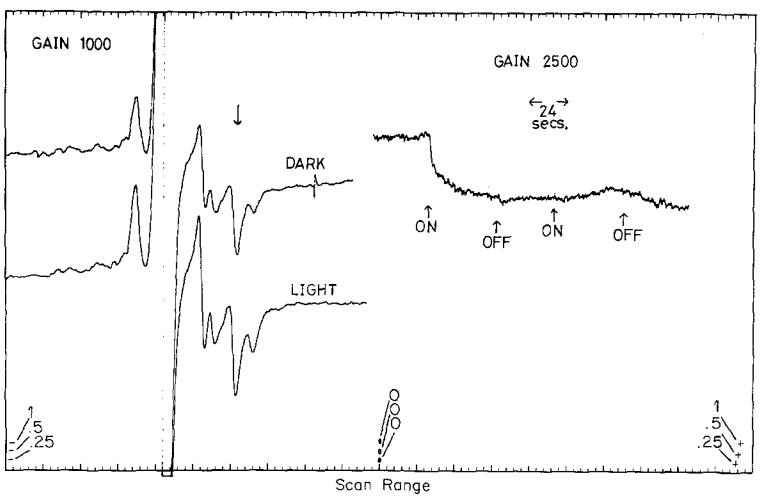

FIG.4 The effect of illumination on the EPR signal of PSI particles poised at - 560 mV. LEFT. EPR spectrum in the g = 2.00 region. RIGHT. Time course of the change in the g = 1.89 signal. Instrument settings as in Fig.1. Gain as indicated.

of Bolton et al[8], that the iron-sulphur centres are not the primary electron acceptor of PSI, but that an unknown component is the primary electron acceptor.

On the basis of redox potential titrations of photosystem I particles, we have suggested[6] that there are two iron-sulphur centres in the primary electron acceptor complex. Centre A with signals at g = 2.05, 1.94, and 1.86 and Centre B with signals at g = 2.05, 1.92, and 1.89. Centre A has $Em_{10.0}$ = -550 mV, and Centre B $Em_{10.0}$ = -590 mV as measured in samples poised at 298°K and then frozen. In samples frozen without dithionite, illumination at low temperature results in the reduction of Centre A only, as would be expected from the redox potential, with a quantitative relationship between the amount of Centre A reduced and P700 oxidized[3]. It is difficult to demonstrate the photoreduction of Centre B at low temperature. We have now demonstrated the photoreduction of Centre B at low temperatures in samples poised so that Centre A is reduced before freezing. Fig.4 shows the effect of illumination on such a sample. The extent of reduction of Centre B is much less than that obtained on illumination at room temperature in the presence of dithionite. The reason for this is not known at present. However, this experiment shows that Centre B as well as Centre A is closely associated with the primary reactions in PSI and that its reduction is coupled to the irreversible photooxidation of P700.

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