DETERMINATION OF THE OXIDATION–REDUCTION POTENTIAL OF THE BOUND IRON-SULPHUR PROTEINS OF THE PRIMARY ELECTRON ACCEPTOR COMPLEX OF PHOTOSYSTEM I IN SPINACH CHLOROPLASTS

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Received 6 October 1974

1. Introduction

Malkin and Bearden [1] showed that spinach chloroplasts contain an EPR-detectable component, which can be photoreduced at low temperatures. Further experiments showed that this component is concentrated in photosystem I particles and is photoreduced by far red light [2,3]. Using varying conditions for photoreduction of the acceptor we were able to show [3] that two EPR detectable components could be detected, both of which had the characteristics of iron-sulphur centres. We further suggested that the two components might be two active centres of a single protein.

More recently, Ke et al. [4] have measured the intensities of the EPR signals of these components as a function of redox potential in digitonin prepared photosystem I particles. They found that these components have extremely low redox potentials. Their interpretation of the data was that there are at least three species of iron-sulphur centres present; and that each of these reductions involved a two-electron change. This result implies that the groups must represent a novel type of redox carrier, since all known iron-sulphur centres accept electrons one at a time. Even if a protein contains two such centres, and these accept electrons individually and independently, one would expect redox behaviour characteristic of one-electron changes [5].

In this paper, we present the results of potentiometric titrations of the components in photosystem I particles prepared from spinach chloroplasts by French press treatment. Our results are broadly in agreement with those of Ke et al. [4] but appear to be more consistent with one-electron transfer processes. In addition we would like to point out that the data can be explained in terms of a simpler hypothesis, that there are just two iron-sulphur centres present, if it is assumed that these centres interact with each other during reduction.

2. Materials and methods

Broken washed spinach chloroplasts were prepared from market spinach by the procedure of Whatley and Arnon [6]. Subchloroplast photosystem I particles at a concentration of 4–6 mg chlorophyll/ml were prepared from the chloroplasts by the procedure of Sane et al. [7] using the French Press as described previously [3].

Oxidation–reduction potentiometry was carried out in an apparatus similar to that described by Dutton [8]. The mixture of mediators used, and the method

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of EPR measurements, were as described by Cammack [9]. In order to achieve sufficiently low potentials all of the results presented in this paper were obtained at pH 10.0. Only partial reduction of the centres was observed at pH more acidic than 9.5. The pH was monitored by a glass electrode in the titration vessel and adjusted if necessary with NaOH. The titrations were carried out at 25°C in dim green light and the samples were allowed to stand in total darkness for 30 sec before freezing in the dark; all subsequent operations performed in the dark. Spectra were measured within 24 hr of sample preparation to avoid possible deterioration of signal intensity on storage.

3. Results

Fig. 1 shows the behaviour of the EPR signals, in the $g = 2$ region, of photosystem I particles as a function of the redox potential at which the particles are poised. The signal at $g = 2.00$ is a free radical due to the reduced mediators. During the first stage of reduction (fig. 1b), signals at $g = 2.05$, 1.94 and 1.86 appear, at lower potentials (fig. 1c), signals at $g = 1.92$ and 1.89 also appear, at the lowest potential (fig. 1d) the signal at $g = 1.89$ is most intense, and that at $g = 1.86$ is no longer visible. These results which were similar to those observed in whole chloroplasts, are in agreement with those reported for digitonin-prepared particles [4].

Fig. 2 shows redox titrations of each of the components of the spectrum. The line at $g = 2.05$ shows an increase during both stages of reduction, indicating that both high and low potential components contribute to it. The signal at $g = 1.86$ might be due to a third component, as previously proposed [4]. However, we feel that the similarity of the potentials for its appearance and disappearance to those for the other signals, may not be a coincidence, and that this line is a component of the higher potential component, which either broadens or undergoes a shift on reduction of the lower potential component, so that it is inseparable from the line at $g = 1.89$. A shift of this type is known to occur in the eight-iron bacterial ferredoxins, such as that from *Veillonella alcalescens* [11], where a low-field line is seen on partial reduction which moves to higher field when the second centre is reduced. Such a shift implies interaction between the two iron-sulphur centres; in the case of the bacterial ferredoxins, the

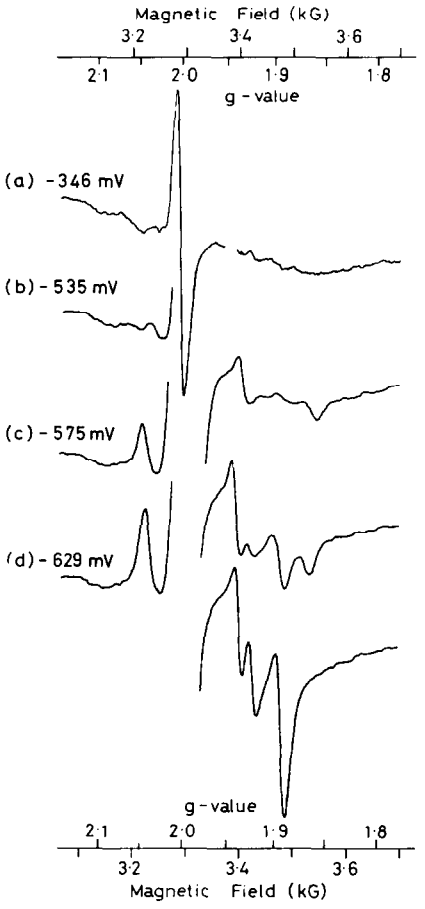

Fig. 1. EPR spectra of subchloroplast particles, 5.0 mg chlorophyll/ml, poised at the potentials shown. Spectra were recorded at 15°K at the following instrument settings: microwave power, 20 mW; frequency 9.22 GHz; modulation amplitude 10 G.

two centres are components of the same protein, and it has been suggested that the change in lineshape is due to spin–spin interactions [12], though conformational changes may play a part.

We therefore propose the following assignments for the EPR signals of the two components, which we will call A and B respectively. Centre A gives rise to an EPR signal in the reduced state with $g_x = 2.05$, $g_y = 1.94$; g_x is 1.86 when the associated centre B is oxidised, and approximately 1.89 when its centre B is reduced. Centre B has $g_z = 2.05$, $g_y = 1.92$ and $g_x = 1.89$.

Theoretical redox-titration curves were calculated by considering a system of two reducible components,

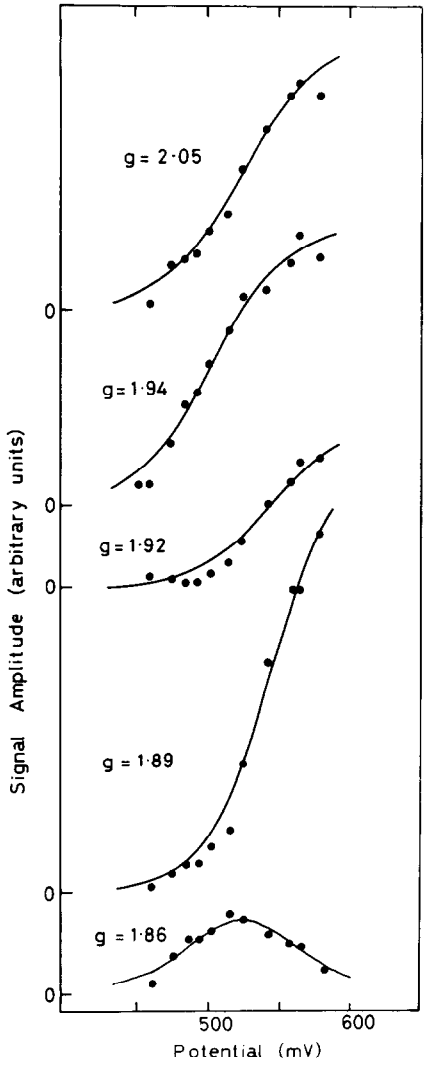

Fig. 2. Variations with redox potential of the various features of the EPR spectrum, with curves calculated as described in the text. The size of the signals at g = 2.05, 1.89 and 1.86 was measured by the height from the oxidised baseline; the signal at g = 1.86 was corrected for the gaussian lineshape of the signal at g = 1.89. The signals at g = 1.94 and 1.92 were estimated from peak-to-peak heights.

A and B, with mid-point potentials E_A and E_B. The reduced forms, A′ and B′, give rise to EPR spectra. At any redox potential the four states of the system, namely AB, A′B, AB′ and A′B′, will reach an equilibrium determined by the Nernst equation. Using the assignments given above for the components of the EPR spectrum, the expected redox behaviour of these components can be estimated. It is assumed that the signal at g = 1.86 belongs to A′B, and shifts to g = 1.89 in A′B′. The exact value of its contribution to the g = 1.89 signal has only a small effect on the shape of the titration curve. The values of E_A, E_B and the intensities of the various components of the signal were adjusted to give the best fits to the data. The curve fitting shows that the redox titration data are consistent with the proposed assignments.

The values used in fig. 2 were E_A = −553 mV, and E_B = −594 mV. These values are lower than the values (−530 and < −580 mV) previously reported [4], but the differences are probably within experimental error. The uncertainty in our values introduced by curve fitting is about ± 5 mV. The overall estimated error in the measurements is about ± 20 mV.

The curves in fig. 2 are drawn assuming that A and B are independent one-electron accepting groups which is the expected behaviour for iron-sulphur centres. However, there is an interesting possibility that there is co-operation between the subunits, so that reduction of one centre makes the mid-point potential of the other less negative. This has the effect of giving an apparent value of n in the Nernst equation somewhat greater than 1, but less than 2. An improved fit to the data was obtained using this assumption, but it is not clear whether this was due to the introduction of another free parameter into the calculation.

In summary, we propose that the redox titration data are consistent with the existence of two-membrane bound iron-sulphur centres in photosystem I, which are closely associated with each other. It should be noted however, that these experiments cannot determine whether either of these centres is the primary acceptor of electrons from P-700, or not.

Acknowledgements

This work was supported in part by grants from the Science Research Council.

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