THE IRON–SULPHUR CENTRES OF AEROBICALLY-GROWN *ESCHERICHIA COLI* K12

An electron paramagnetic resonance study

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1. Introduction

A $g = 1.94$ iron–sulphur centre signal in EPR spectra of *Escherichia coli* was first noted in [1]. Since that time, the study of iron–sulphur proteins in this organism surprisingly has been rather neglected. Membrane particles from *E. coli* contain considerable amounts of non-haem iron, although perhaps only a fraction is associated with functional iron–sulphur centres. The presence of several iron–sulphur proteins has been suggested, based upon:

(i) Their differential reactivity with *o*-phenanthroline [2,3] and the more lipophilic iron chelator bathophenanthroline [4];

(ii) The detection of various $g = 1.94$ signals in EPR spectra of membrane preparations at 77 K [1,5,6] and at 12 K [7].

Here we show, using EPR, that membrane fragments derived from aerobically-grown *E. coli* contain ⩾4 iron–sulphur centres, 3 of which are similar in many respects to those of the succinate dehydrogenase of eukaryote mitochondria [8] and of other prokaryotes (e.g. [9]). Two ferredoxin-type centres with prominent $g = 1.94$ resonances and a further centre with a resonance at $g = 1.875$ and 2.05 are seen (all paramagnetic in the reduced state), together with a HiPIP-like signal (paramagnetic in the oxidized state). The $g = 1.94$ and the HiPIP centres all have midpoint redox potentials close to those observed in reconstituted mammalian succinate dehydrogenase [8,10] and in this enzyme from *Rhodopseudomonas sphaeroides* [9]. We also find a copper centre with a midpoint potential of ~+100 mV.

2. Materials and methods

2.1. *Growth of cells*

Two strains of *E. coli* K12 were used. Strain EMG-2 is a prototroph and was supplied by Dr M. Peacey, University of Edinburgh. Strain A 1002 (K-12 Y *mel ilv⁻ lac* I⁻ *met* E⁻) was kindly given by Dr B. A. Haddock, University of Dundee. Either strain was grown in a mineral salts medium modified from that in [11] supplemented with vitamin-free casamino acids (0.1%, w/v), either glycerol or sodium succinate (each 0.5%, w/v) and, for strain A1002 [12], isoleucine, valine and methionite (each at 0.02 g/l). Cultures (5–20 l) were aerated by vigorous sparging with sterile air. Essentially similar results were obtained with either strain and with glycerol or succinate as major carbon sources.

2.2. *Harvesting and breakage*

Cells were harvested in the late exponential phase of growth [12,13]. Cells were washed twice with growth medium and then disrupted using either sonication [7] or two passages through a French pressure cell at ~120 MPa. Membrane particles (electron-transport particles) were prepared by differential centrifugation essentially as in [12,13].

2.3. *Redox potentiometry*

Oxidation–reduction midpoint potential were determined essentially as in [14] as applied to EPR

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[15]. EPR measurements were made using a Varian E109 spectrometer equipped with an Air Products liquid helium transfer line (LTD-3-110) [8]. Quantitation of the signals was performed against a Cu(II)–EDTA standard.

3. Results and discussion

3.1. *The ferredoxin-type iron–sulphur centres detectable in membrane particles from aerobically-grown* E. coli

Fig.1 shows the paramagnetic components detectable at 10 K in NADH (A) and lactate-reduced (B) membrane preparations. A number of overlapping lines attributable to iron–sulphur centres are seen after reduction with these substrates and also with succinate (not shown) and dithionite (fig.2). The major resonance is centred at $g = \sim 1.94$ with a g_z signal at ~2.03. This signal is considerably more intense after reduction by NADH (fig.1A) than by lactate (fig.1B). In both preparations, a further signal is seen at $g = 1.875$, together with other less well-resolved components at higher field values. Fig.2 shows spectra obtained of dithionite-reduced membranes at various sample temperatures. The $g = 1.875$ resonance is seen only at $\lesssim$20 K; the signal was not saturated at 8 K. In contrast, the $g = 1.94$ signal is seen at $\geqslant$50 K. Also seen at higher temperatures are resonances at $g = 2.01$, 2.015, 2.05 of unknown origin and the g_z component at 2.027 of the $g = 1.94$ signal.

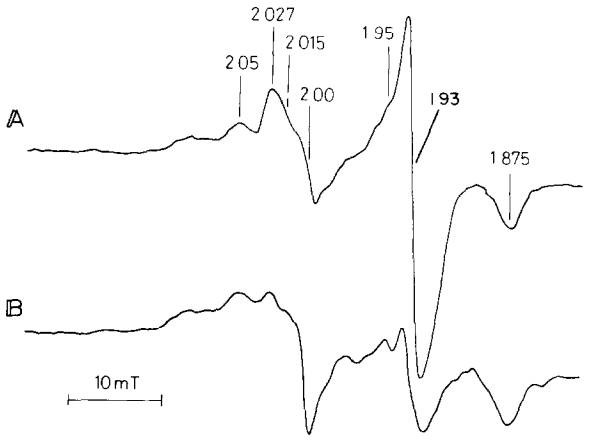

Fig.1. EPR spectra of *E. coli* membrane particles reduced with 5 mM NADH (A) or 1.5 mM lactate (B). EPR conditions were: temp. 10 K; receiver gain, 6.3×10^3; microwave power, 10 mW; modulation amplitude, 10^{-3} T; microwave frequency, 9.05 GHz.

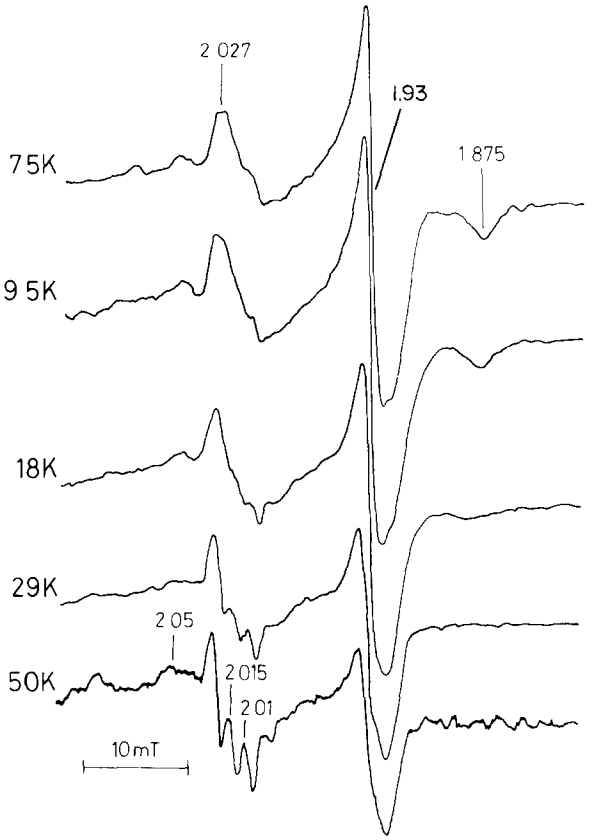

Fig.2. EPR spectra, taken at various temperatures, of *E. coli* membrane particles reduced with dithionite. EPR conditions were: receiver gain, 3.2×10^3; microwave power, 10 mW; modulation amplitude, 10^{-3} T; microwave frequency, 9.05 GHz. Temperature of the sample was varied as shown on the left of the figure.

A $g = 2.05$ signal is observed in mitochondrial systems and originates from the N-2 iron–sulphur centre, the component with highest midpoint potential in the NADH–UQ reductase segment. In the mitochondrial centre N-2 normally has signals at $g_{\parallel} = 2.05$ and $g_{\perp} = 1.93$ [16] the latter giving a sharp trough at 1.92. Such a $g = 1.92$ signal is not observed in the *E. coli* membranes; however if it were broader than normal, as has been observed in some plant mitochondria [17], it is possible that the signal is hidden in the 1.94 signal and masked by centre S-1 in the redox titrations. The 2.05 signal seen in fig.1,2 may be overlapped signals from different centres having slow and fast relaxation properties. Quantitation of the ferredoxin

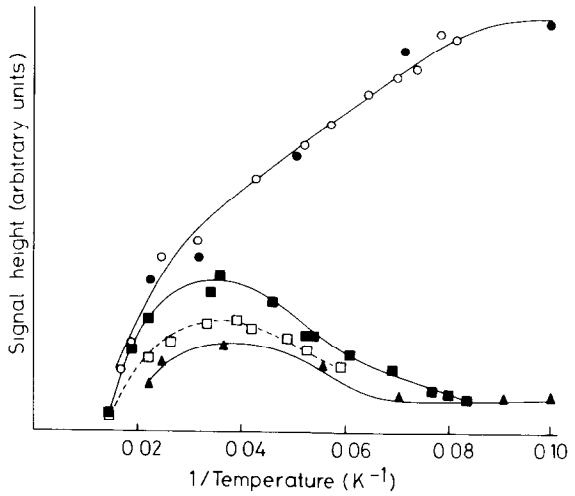

Fig.3. Temperature dependence of the g = 1.94 signal heights (measured from peak to trough) in samples of membrane particles reduced by dithionite (○), 5 mM NADH (●), 12.5 mM lactate (▲) or endogenous substrates (□), and poised at −100 mV (■). EPR conditions were: microwave power, 10 mW; modulation amplitude, 1 × 10^{-3} T; microwave frequency, 9.05 GHz.

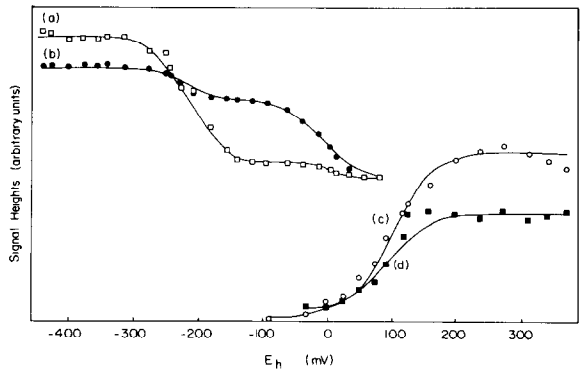

Fig.4. Potentiometric analysis of the EPR-detectable components in *E. coli* membrane particles. Curves (a,b) show the results at two temperatures of a redox titration of the g = 1.94 signal. Curves (c,d) show the behaviour of the HiPIP centre (g = 2.02 peak height) and the copper, respectively. The redox titration was performed in 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2 ethane-sulfonic acid (Hepes) buffer (pH 7.0), containing 10 mM EDTA. Dibromophenolindophenol, 2-hydroxy-1,4-naphthoquinone, 1,4-naphthoquinone, duroquinone, indigodisulphonate, indigotetrasulphonate, 2,3,5,6-tetramethylphenylenediamine and *N*-methylphenazonium methosulphate (all at 40 μM) were used in the higher potential range. Similar concentrations of methyl viologen, benzyl viologen, phenosafranine, anthraquinone-2-sulphate, 2-OH 1,4 naphthoquinone, the indigo-sulphonates and *N*-methylphenazonium methosulphate were used for the lower potential range. EPR conditions were: temp. (a) 10 K, (b), 30 K, (c) 8 K, (d) 30 K; microwave power, 10 mW; modulation amplitude, 1 × 10^{-3} T; microwave frequency, 9.05 GHz.

content of dithionite reduced membranes gave ~0.16 nmol spins/mg protein.

The g = 1.94 signals observed after reduction by various physiological substrates or dithionite have different temperature profiles as shown in fig.3, where the signal height is plotted as a function of reciprocal sample temperature. The ferredoxin-type centres reducible by dithionite or by NADH exhibit more rapid relaxation rates than does the g = 1.94 signal reducible by lactate or by endogenous substrates. There is not evidence for an N-2 type centre hidden by the g = 1.94 absorption in these profiles. Also shown is the temperature dependence of the g = 1.94 signal in a sample poised at −100 mV. The centre reducible by lactate and endogenous substrates corresponds to the higher potential ferredoxin of the 2 major components resolved in the redox titration of fig.4, because of the similar temperature profile obtained for these centres and that obtained at redox potentials where only the higher potential component is reduced. The 2 major ferredoxin components resolved (fig.4) have $E_{m,7}$ values of ~−20 mV and −220 mV, respectively. Both dithionite and NADH are able to reduce the low potential ferredoxin. The low potential component is from NADH dehydrogenase or from centre S-3 or from both; this remains to be studied more quantitatively. Endogenous substrate and lactate do not reduce the low potential centre either for thermodynamic reasons ($E_{m,7}$ pyruvate/lactate; −190 mV) or mechanistic reasons. The g = 1.875 and 2.05 signals shown in fig.1,2 titrates with a midpoint potential in the vicinity of −250 mV (results not shown). Alkaline treatment of the membranes (washing in pH 10 borate buffer) did not remove the iron–sulphur centres from the membrane, in contrast to the removal of mammalian succinate dehydrogenase from mitochondrial particles [18].

3.2. *Those centres that are observed in the oxidized form; HiPIP and cupric ion*

Spectra of oxidized membrane particles taken at two temperatures are shown in fig.5. The upper spectrum (8 K) shows a sharp relatively-symmetrical absorption characteristic of some mitochondrial HiPIP signals [10,18–20]. Similar signals have been

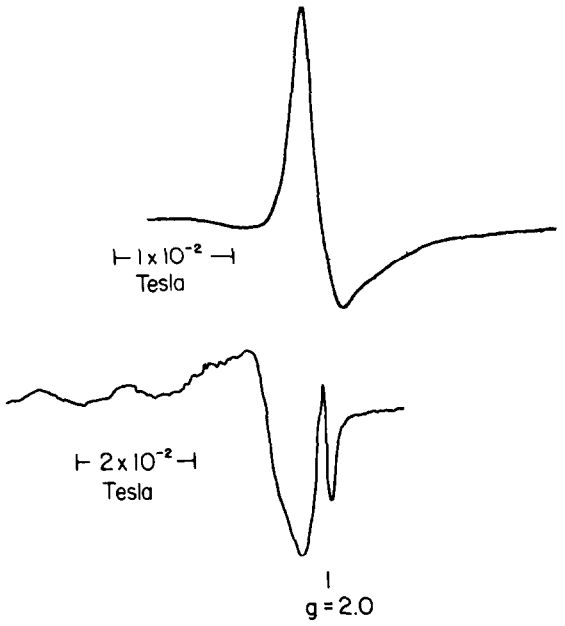

Fig.5. EPR spectra of oxidized membrane particles from *E. coli*. In (a) EPR conditions were: temp. 8 K; microwave power, 10 mW; modulation amplitude, 1.6×10^{-3} T; microwave frequency, 9.05 GHz. In (b) these values were: 30 K; 0.1 mW; 2×10^{-3} T; 9.05 GHz.

described in bacterial systems [9,21,22]. The signal is highly temperature-dependent, being observed only at $\lesssim$25 K and increasing in intensity at <7 K. The lower spectrum in fig.5 (30 K) shows predominantly copper at an est. 0.09 nmol/mg protein. The signal persisted after washing the membranes with 50 mM EDTA. These results are in contrast [23] where Cu^{2+} was not detected by EPR at 77 K in membranes from *E. coli* and 7 other bacteria containing widely different cytochrome oxidases. EPR-detectable Cu^{2+} was concluded [23] to represent <2.5% of the total copper measured by atomic absorption spectroscopy, but in the samples, the Cu^{II} may have been reduced to Cu^{I} by endogenous respiration.

4. Conclusion

Membrane particles derived from *E. coli* K12 grown aerobically on glycerol or succinate as carbon source contain the same major iron–sulphur centres. These comprise two ferredoxins seen at $g = 1.94$ and at least one at $g = 1.875$ and 2.05 and a HiPIP. The properties of the centres bear a close resemblance to the two ferredoxins (S-1, S-2) and HiPIP (S-3) which make up (along with FAD) mammalian succinate dehydrogenase. The presence of additional low potential clusters analogous to NADH-dehydrogenase components are also indicated. The presence of the copper is more problematic; there is no indication of what role it may play and it is possible that the absorption is due to adventitious copper. However, its high concentration, midpoint potential and stability towards washing by 50 mM EDTA indicate otherwise.

Acknowledgements

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