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Ubisemiquinone in Membranes from *Escherichia coli*

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The e.s.r.‡ spectra of respiratory membranes from mitochondria show an asymmetric signal with the major component in the region of $g = 1.9$ that has been attributed to 'non-haem iron' (Sands & Beinert, 1960; see also Beinert & Palmer, 1965). A symmetrical signal due to an organic radical ($g \simeq 2.00$) has also been found in respiratory membranes (Sands & Beinert, 1960), but has not been definitely assigned owing to the lack of hyperfine structure in protein-bound radicals (see Beinert & Palmer, 1965). Two possible sources of the latter signal have been suggested, namely the semiquinones of flavin and ubiquinone (see Beinert & Palmer, 1965).

As part of a study of the electron-transport system of *Escherichia coli* K12, the e.s.r. spectra of membrane suspensions were recorded. Membranes were isolated by ammonium sulphate fractionation (Cox, Snoswell & Gibson, 1968) from *E. coli* K12 strain AN 62 (Cox, Young, McCann & Gibson, 1969), which possessed a normal respiratory system. The e.s.r. spectrum of these membranes is depicted in Fig. 1(*a*). The spectrum consists of a symmetrical signal with $g = 2.003 \pm 0.001$ and with a peak-to-peak width of 12G. The *g* value, symmetry, peak-to-peak width and the saturation at relatively low power levels are consistent with an organic free radical of the semiquinone type being the source of the signal (Beinert & Palmer, 1965). The maximum value of the signal corresponded to about 2% of the total ubiquinone present and was reached at different times depending on the membrane preparation used. The signal was always relatively intense initially, but was removed by continued incubation in the e.s.r. tube at 0°C for 24h, to be replaced by an asymmetric signal with two peaks at *g* values of 2.02 and 1.92 (Fig. 1*b*). These two peaks are part of the same signal, as they appeared simultaneously at a constant intensity ratio and, in addition, they behaved identically on power saturation. The signal is similar to the '$g = 1.94$' signal previously found under reducing conditions in preparations from *E. coli* K12 and attributed to non-haem iron (Nicholas, Wilson, Heinen, Palmer & Beinert, 1962). As expected for e.s.r. signals from non-haem iron (see Beinert & Palmer, 1965), the signal shown in Fig. 1(*b*) saturated less readily than the signal due to the radical and was more sensitive to recording temperature. The addition of the substrates NADH (1 μmol) or D-lactate (5 μmol) resulted in similar, but more rapid, spectral changes to those described above, the whole process being completed within a few minutes at 0°C. The increased rate is consistent with the relative times taken for anaerobic conditions to be established, as estimated with an oxygen electrode (G. B. Cox, unpublished work).

The organic radical found in strain AN 62 (Fig. 1*a*) may be due to the semiquinone form of flavin, vitamin K or ubiquinone. Therefore membrane preparations from a strain of *E. coli* K12 (strain AN 59) unable to form ubiquinone (Cox *et al.* 1969) were examined. The radical could not be detected in these preparations (Fig. 1*c*) regardless of the time of incubation. However, on the addition of ubiquinone (Q-1), the free-radical signal was observed (Fig. 1*d*), but at a lower intensity than that present in membranes from strain AN 62. Continued incubation at 0°C for 24h again removed the radical signal, which was replaced by the signal attributed to non-haem iron (Fig. 1*e*). The addition of substrate once again accelerated the spectral changes. The non-haem-iron signal could also be observed in membranes from strain AN 59, in the absence of added ubiquinone, either on prolonged incubation or after the addition of substrate.

It would therefore seem that the organic radical observed in respiratory membranes of *E. coli* is probably due to the presence of ubisemiquinone. However, because of the lack of hyperfine structure in the signal, the possibility of another radical derived from ubiquinone, e.g. the chromanoxyl radical (see Kohl, Wright & Weissman, 1969), cannot be dismissed.

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‡ Abbreviation: e.s.r. electron spin resonance.

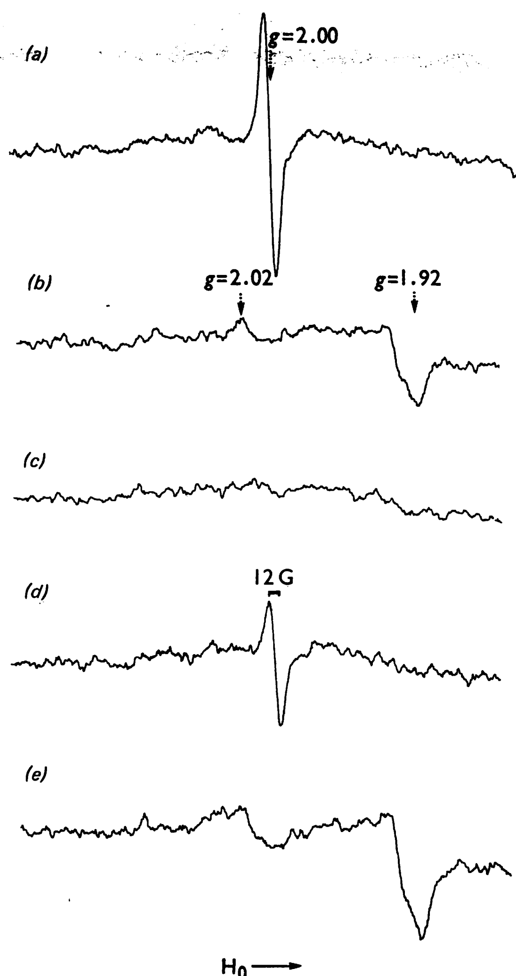

Fig. 1. E.s.r. spectra of membranes from *E. coli* K12. (*a*) Membranes from strain AN 62 after 30 min incubation at 0°C; (*b*) membranes as for (*a*) but after 24 h incubation at 0°C; (*c*) membranes from strain AN 59 frozen immediately after mixing at 0°C; (*d*) membranes as for (*c*), but 0.4 μmol of ubiquinone (Q-1) added previously to the e.s.r. tube; the sample was frozen immediately after mixing at 0°C; (*e*) the same components as for (*d*), but incubated for 24 h at 0°C. The e.s.r. samples were prepared as follows. To an e.s.r. tube was added 0.1 ml of buffer (15 mM-sodium–potassium phosphate, pH 7.4, containing 1.9 mM-$MgCl_2$) and, where indicated, 10 μl (0.4 μmol) of ubiquinone (Q-1). The membrane preparation (0.1 ml containing 10 mg of protein) was then added and mixed gently with a syringe. Spectra were recorded after the samples had been frozen in liquid N_2. Mixing and freezing the contents of the tube took a minimum time of 30 s. The *X*-band spectra were recorded as first-derivative spectra with a Varian V-4501 spectrometer. The recording conditions were as follows: temperature, 77°K; microwave frequency, 9.05 GHz; modulation frequency, 100 kHz; modulation level, 3.78 G peak-to-peak; power level, 61 mW; integrating time constant, 1 s; gain setting, ×1000; scanning rate, 500 G/10 min. The concentrations of unpaired spins were calculated by double integration by comparison with a standard Varian dilute pitch sample.

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