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The orientation of iron–sulphur clusters in membrane multilayers prepared from aerobically-grown *Escherichia coli* K12 and a cytochrome-deficient mutant

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1. Membrane particles prepared from ultrasonically-disrupted, aerobically-grown *Escherichia coli* were centrifuged on to a plastic film that was supported perpendicular to the centrifugal field to yield oriented membrane multilayers. In such preparations, there is a high degree of orientation of the planes of the membranes such that they lie parallel to each other and to the supporting film. 2. When dithionite- or succinate-reduced multilayers are rotated in the magnetic field of an e.p.r. spectrometer, about an axis lying in the membrane plane, angular-dependent signals from an iron–sulphur cluster at $g_x = 1.92$, $g_y = 1.93$ and $g_z = 2.02$ are seen. The $g = 1.93$ signal has maximal amplitude when the plane of the multilayer is perpendicular to the magnetic field. Conversely, the $g = 2.02$ signal is maximal when the plane of the multilayer is parallel with the magnetic field. 3. Computer simulations of the experimental data show that the cluster lies in the cytoplasmic membrane with the g_y axis perpendicular to the membrane plane and with the g_x and g_z axes lying in the membrane plane. 4. In partially-oxidized multilayers, a signal resembling the mitochondrial high-potential iron–sulphur protein (Hipip) is seen whose $g_z = 2.02$ axis may be deduced as lying perpendicular to the membrane plane. 5. Appropriate choice of sample temperature and receiver gain reveals two further signals in partially-reduced multilayers: a $g = 2.09$ signal arises from a cluster with its g_z axis in the membrane plane, whereas a $g = 2.04$ signal is from a cluster with the g_z axis lying along the membrane normal. 6. Membrane particles from a glucose-grown, haem-deficient mutant contain dramatically-lowered levels of cytochromes and exhibit, in addition to the iron–sulphur clusters seen in the parental strain, a major signal at $g = 1.90$. 7. Only the latter may be demonstrated to be oriented in multilayer preparations from the mutant. 8. Comparisons are drawn between the orientations of the iron–sulphur proteins in the cytoplasmic membrane of *E. coli* and those in mitochondrial membranes. The effects of diminished cytochrome content on the properties of the iron–sulphur proteins are discussed.

In contrast with the wealth of information on the e.p.r.-detectable iron–sulphur proteins of mitochondrial respiratory chains, very little is known of the chemistry or function of these components in bacteria. However, based on their reactivity with iron chelators (Bragg, 1974) and the detection of various $g = 1.94$ signals in e.p.r. spectra of membrane preparations at 77K (Nicholas *et al.*, 1962; Hamilton *et al.*, 1970; Hendler & Burgess, 1974) and 12K (Poole & Haddock, 1975), a role for iron–sulphur centres in the respiratory chains of *Escherichia coli* and other bacteria (e.g. Meijer *et al.*, 1977) has been firmly established. Recently, Ingledew *et al.* (1980) have detected four iron–sulphur clusters in aerobically-grown *E. coli* that are similar in many respects to those of succinate dehydrogenase in mitochondria (Beinert *et al.*, 1975; Ohnishi *et al.*, 1976*a,b,c*) and certain other bacteria (Ingledew & Prince, 1977).

In a number of studies of membranous biological

Abbreviation used: Hipip, high-potential iron–sulphur protein.

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structures such as mitochondria (Erecińska *et al.*, 1977; Salerno *et al.*, 1979), submitochondrial particles (Blum *et al.*, 1978*a*), chromatophores (Tiede *et al.*, 1978) and bacterial membrane particles (Poole *et al.*, 1980), it has been shown that the membrane-bound electron and hydrogen carriers are well-oriented in the membrane. The most extensively-studied component is cytochrome *c* oxidase, whose haem normal has been shown to lie in parallel with the mitochondrial membrane plane (e.g. Blum *et al.*, 1978*a*; Erecińska *et al.*, 1979). Iron–sulphur clusters (Erecińska *et al.*, 1978; Salerno *et al.*, 1978; Erecińska & Wilson, 1979; Salerno *et al.*, 1979) also appear to be fixed in the membranes at definite angles relative to the membrane plane.

A general structure–function relationship remains to be elucidated for these proteins, although it is expected that proteins with generally similar function and composition in various organisms will tend to have similar geometries.

In this paper, we describe e.p.r. studies of the orientations of the iron–sulphur proteins found in membranes derived from an aerobically-grown parent strain of *E. coli* K12 and a cytochrome-deficient mutant.

Experimental

Organisms, growth conditions and preparation of membrane particles

Escherichia coli strain A1002 (K12 Y mel *ilv*$^-$,*lac I*$^-$,*met E*$^-$) was grown aerobically in a defined medium containing succinate as carbon source and supplemented with casamino acids, exactly as described by Poole *et al.* (1979). The harvesting and washing of cells (Poole *et al.*, 1979), their disruption by sonication (Poole & Haddock, 1975), and fractionation of the sonicate by differential centrifugation to yield a membrane preparation (previously called 'electron transport particles'; Poole & Haddock, 1974) were all carried out by previously-published methods.

E. coli strain A1004a (K12 Y mel *ato*$^-$,*fad* R^c,*hem* A$^-$,*ilv*$^-$,*lac I*$^-$,*met E*$^-$,*rha*$^-$) is defective in δ-aminolaevulinate synthase (Haddock, 1973). It cannot grow on glycerol or intermediates of the citric acid cycle unless supplemented with δ-aminolaevulinic acid, although it will grow aerobically or anaerobically with glucose as carbon source. It was kindly provided by Dr. B. A. Haddock, University of Dundee, Dundee, Scotland, U.K. The growth procedure closely resembled that of Kemp *et al.* (1975). Cultures were maintained by adding sterile glycerol to a broth culture (see below) to a final concn. of 12.5% (w/v) which was then stored in 3ml aliquots at −20°C. For growth of the strain, a frozen culture was thawed and a drop was added to 20ml of broth that contained, per litre: K_2HPO_4, 4g; KH_2PO_4, 1g; tryptone, 10g; yeast extract, 5g; glucose, 5g; δ-aminolaevulinic acid, 5mg. The final pH was 7.2. Glucose and δ-aminolaevulinic acid were added separately as sterile solutions; glucose was autoclaved and δ-aminolaevulinic acid was sterilized by membrane filtration. The inoculated broth was incubated anaerobically for 24h at 37°C. A portion (0.5ml) of the broth culture was inoculated into 100ml of medium that contained, per litre: K_2HPO_4, 4g; KH_2PO_4, 1g; NH_4Cl, 1g; glucose, 5g; $CaCl_2,2H_2O$, 10mg; trace elements solution, 10ml; vitamin-free casamino acids (Difco), 1g; K_2SO_4, 2.6g; δ-aminolaevulinic acid, 5mg; $MgCl_2,6H_2O$, 0.2g; isoleucine, valine and methionine, 20mg each. The final pH was 7.1–7.2. The trace elements solution is that described by Poole *et al.* (1979).

The inoculated medium, in a 100ml Erlenmeyer flask, was incubated anaerobically at 37°C for 16–20h and was then transferred to 4°C until the result from a screen of the culture for revertants was known. Reversion was checked by spreading 0.1ml portions of culture on a medium of the same composition as the second medium above, except that glycerol (5g/l) replaced glucose as a carbon source and the medium was solidified with agar (15g/l). Growth on glycerol requires a functional electron transport chain; thus, revertants were identified as colonies able to grow on such medium when δ-aminolaevulinic acid was omitted. The above procedures for subculture were effective in minimizing reversion of the mutant. Starter cultures shown to be essentially free of revertants were inoculated into 5 litres of the same medium, containing glucose as carbon source but lacking δ-aminolaevulinic acid. Cells were harvested in the exponential phase of growth, when A_{420} (10mm light path, 1:10 dilution) had reached 0.1. Subsequent procedures were exactly as described above for strain A1002.

Preparation of oriented multilayers of membrane particles

Membrane particles stored at −30°C were rapidly thawed, diluted about 15-fold and homogenized in distilled water. They were centrifuged for 60min at 23000rev./min (70000*g* at $r_{av.}$) onto a circular sheet of Mylar (a plastic film approx. 0.13mm thick). The plane of the Mylar was supported at a normal to the centrifugal force by a specially constructed Perspex insert for the tubes of the swinging bucket SW 25.1 rotor of a Beckman L5-50 ultracentrifuge. The Mylar sheet had previously been washed with detergent and water, and then coated with a film of collodion by dipping in a 1% (w/v) solution in ethanol/diethyl ether (75:25, v/v). After centrifugation, the Mylar sheet and the adhering pellet of particles (0.5–1.0cm diam., 0.5–1.0mm

thick) was drained of excess liquid and allowed slowly and partially to dehydrate by incubating in an atmosphere of 90% relative humidity at 4°C for 48–72h. Membranes in such multilayers are oxidized, as demonstrated by the e.p.r. spectra (see the Results and discussion section).

Electron microscopy of sections of such multilayers, cut perpendicular to the Mylar plane, shows substantial orientation of flattened membranes, parallel to each other and to the Mylar (Poole *et al.*, 1980).

Reduction of membrane multilayers

A few drops of a saturated solution of either $Na_2S_2O_4$ or sodium succinate were added to the stacked multilayers in the e.p.r. tube and allowed to react for about 2min before freezing the sample by immersion in liquid N_2. Alternatively, for particles from strain A1002, an attempt was made to keep substrate-reduced particles anoxic during the preparation of multilayers. Thawed particles in 5mM-Tris/HCl, pH7.4, were made anoxic by adding sodium succinate (25mM final concn.) and subsequently centrifuged in deoxygenated water containing 1mM-succinate. Partial dehydration was carried out at 90% relative humidity in an atmosphere of H_2 and CO_2 in a GasPak Disposable Anaerobic System (BBL, P.O. Box 175, Cockeysville, MD 21030, U.S.A.).

E.p.r. studies

The hydrated multilayer on its Mylar support was sliced into rectangular pieces, each approx. 2mm × 10mm. Usually two or three of these pieces were slipped into a standard e.p.r. tube. The top of the tube was sealed and fitted with a pointer that was parallel to the plane of the Mylar sheets. The tube was mounted in a tubular holder fitted with a protractor, so that the angle of the Mylar in the magnetic field could be read. The experimental arrangement is similar to that shown by Salerno *et al.* (1979) except that a standard 4mm-diam. quartz e.p.r. tube was used. Field angles given are those between the direction of the magnetic field and the normal to the Mylar film; that is, the angle is given as 90° when the plane of the Mylar is parallel to the magnetic field. Referring to Fig. 1 of Blum *et al.* (1978*a*), the angle quoted is shown as ω. E.p.r. spectra were taken on a Varian E109 spectrometer. Details of sample cooling and temperature control have been described by Blum *et al.* (1978*a*).

Data were analysed with the aid of a computer program (Blum *et al.*, 1978*b*). The e.p.r. spectrum of membrane-bound chromophores in oriented membrane multilayers can be simulated by a model that assumes a fixed orientation of the paramagnetic centre relative to the membrane normal, and no order with respect to rotation about the membrane normal. The program models mosaic spread (wobble of the chromophore in the direction of the membrane normal) by using a Gaussian distribution of chromophore orientations (see Fig. 1 in Blum *et al.*, 1978*b*).

Protein determinations

An estimate of the particle protein present on Mylar films was obtained by treating small slices (about 2–3 mm^2) of the Mylar and attached particles with 0.5M-NaOH for 7min at 95–100°C. The digested protein was assayed by using the method of Lowry *et al.* (1951) with dry bovine plasma albumin as standard. The multilayers described here contained 22–34 μg of protein/mm^2.

Results and discussion

Signals observed in membrane multilayers reduced in situ with dithionite or succinate

Reduction of membrane multilayers in the e.p.r. tube by adding either dithionite (Fig. 1*a*) or succinate (Fig. 1*b*) results in the appearance of the same major e.p.r.-detectable signals. In both cases, an iron–sulphur cluster with principal values, $g = 1.92$, $g = 1.93$ and $g = 2.02$ is seen. In unoriented particle suspensions, at least two ferrodoxin-type components with these g values have been resolved potentiometrically having mid-point potentials of approx. −20mV and −220mV respectively (Ingledew *et al.*, 1980). The component reducible by dithionite exhibited a more rapid relaxation rate than signals with similar g values elicited by reduction with lactate or endogenous substrates, and was tentatively equated with the component of lower redox potential.

Fig. 1 shows that the amplitudes of signals due to iron–sulphur clusters in either dithionite- or succinate-reduced particles exhibit the same dependence on the angular variation of the multilayer within the magnetic field. At a field angle of 0° (i.e. when the plane of the Mylar makes a right angle with the magnetic field), the $g = 1.93$ signal exhibits its maximal amplitude, whereas the converse is true for the $g = 2.02$ signal. A similar, but slight, angular dependence of a $g = 1.93$ signal was noted in a preliminary report of iron–sulphur clusters in mitochondrial multilayers by Erecińska *et al.* (1978). The apparent angular variation of the $g = 1.93$ signal with respect to its position and amplitude can be understood as a sum of two signals: a $g_x = 1.92$ signal maximum at a field angle of 90° and a $g_y = 1.93$ signal described above. These two signals are partially overlapped. The signal at $g = 2.01$ seen in the succinate-reduced sample at 40K (Fig. 1*b*) is also seen when a dithionite-reduced sample is examined at this temperature (result not shown). Its intensity is too low for any angular dependence to be

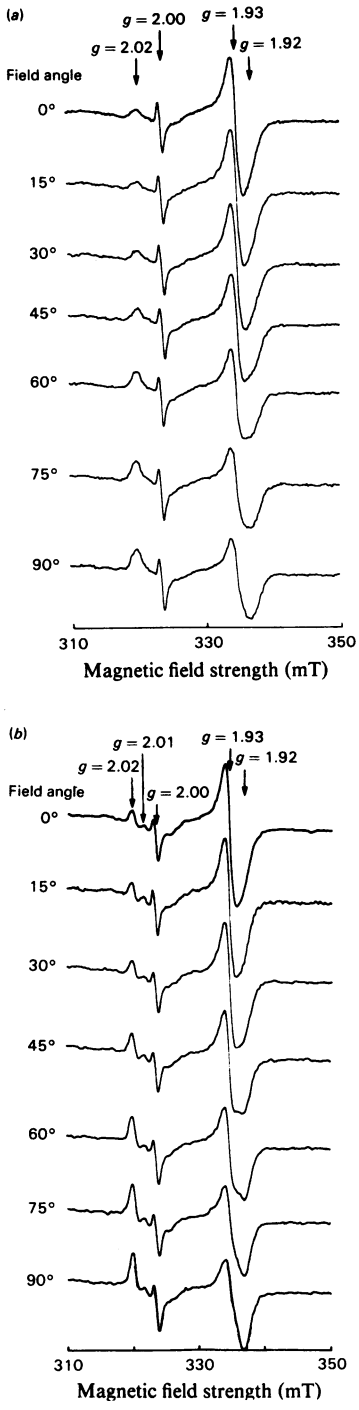

Fig. 1. *Angular dependence of the e.p.r. spectra of reduced membrane multilayers from E. coli*
Membrane multilayers prepared from the parental strain of *E. coli* were reduced in the e.p.r. tube with a solution of $Na_2S_2O_4$ (*a*) or sodium succinate (*b*). The field angle is defined as the angle that the applied magnetic field makes with the normal to the multilayer plane. Prominent features of the spectrum are labelled with their *g* values. The sample temperature was 20K in (*a*) and 40K in (*b*). Other e.p.r. conditions were similar in (*a*) and (*b*) and were: incident microwave power, 10mW at 9.06GHz; modulation amplitude, 1mT at 100KHz; scan rate, 100mT/min with a 0.128s time constant.

assessed. The isotropic signal at $g = 2.00$ is from flavin and serves to demonstrate that the apparent angular dependence of the iron–sulphur signals is not due to drift in the spectrometer response, temperature or other trivial factors.

The experimental e.p.r. spectra (Fig. 1) can be well simulated by using a computer program (Fig. 2). The simulation is optimal when the g_y axis ($g = 1.93$) of the cluster is assumed to lie along the normal to the membrane multilayer and the g_x and g_z axes are assumed to lie in the multilayer plane. The extent of disorder (mosaic spread) was simulated by assigning a value of 35° to the cone subtended by wobble of the chromophore with respect to the applied magnetic field (Fig. 1 of Blum *et al.*, 1978*b*). These signals, therefore, bear not only a spectral and potentiometric resemblance to centres S-1 and S-2 in mitochondrial succinate dehydrogenase (Ohnishi, 1973; Ohnishi *et al.*, 1973; Ohnishi *et al.*, 1976*a*), but also an organizational equivalence, since the g_z (near $g = 2.025$) signal of binuclear [2Fe–2S] iron–sulphur clusters and Rieske's centre (Rieske *et al.*, 1964) in mitochondrial membranes is maximal when the magnetic field is parallel to the plane of the multilayer (Salerno *et al.*, 1979).

Signals observed in anoxically-handled membrane multilayers

The problems encountered in reducing (or preparing liganded derivatives of) chromophores in partially-dehydrated membrane multilayers have been discussed by Erecińska *et al.* (1978). They include the rather slow penetration to their reaction sites of added aqueous reagents and the dangers of disturbing the orientation, either directly, or from decomposition or reaction products of the added solutions. For these reasons, an attempt was made (described in the Experimental section) to maintain anoxia during preparation and handling of multilayers previously reduced with a physiological substrate. Such multilayers proved to be only partially reduced as revealed by their e.p.r. spectra. The low-field portion of the spectrum (60–300mT) showed the contribution of low-spin ($g = 3.0$, $g = 2.25$) and high-spin ($g = 6.0$) cytochromes (results not shown). Marked orientation of these cytochromes was evident, and has been described in detail elsewhere (Poole *et al.*, 1980): their importance for the present purpose is that they are

indicative of incomplete reduction of the multilayers. A prominent isotropic $g = 4.3$ signal (i.e. whose intensity is independent of field angle) was seen that is thought to be due to ferric iron in a field of low symmetry, i.e. no longer specifically associated with protein (Blumberg, 1967).

In the region of the spectrum near $g = 2$ (Fig. 3), low-temperature observation reveals a sharp, relatively-symmetrical absorption. This is similar to oxidized centre S-3, a mitochondrial Hipip (Ohnishi *et al.*, 1974; Ohnishi *et al.*, 1976*b*,*c*) and the tetranuclear [4Fe–4S] high-potential protein of *Chromatium* (Dus *et al.*, 1967). Similar signals have been described in other bacteria (e.g. Meijer *et al.*, 1977). The angular dependence of the signal in Fig. 3 is similar to that observed in fully air-oxidized multilayers (spectra not shown). The $g = 2.02$ peak has greatest amplitude when the field angle is 0°. A similar orientation has been described for the $g_z = 2.02$ signal of centre S-3 in multilayers prepared from submitochondrial particles (Salerno *et al.*, 1979). Computer simulations described by these authors demonstrate that such an angular dependence of the e.p.r. signals results from a centre having its $g_z = 2.02$ axis perpendicular to the membrane plane.

When the receiver sensitivity was increased, smaller signals could be seen on either side of the Hipip-like signal. Spectra recorded at high gain and

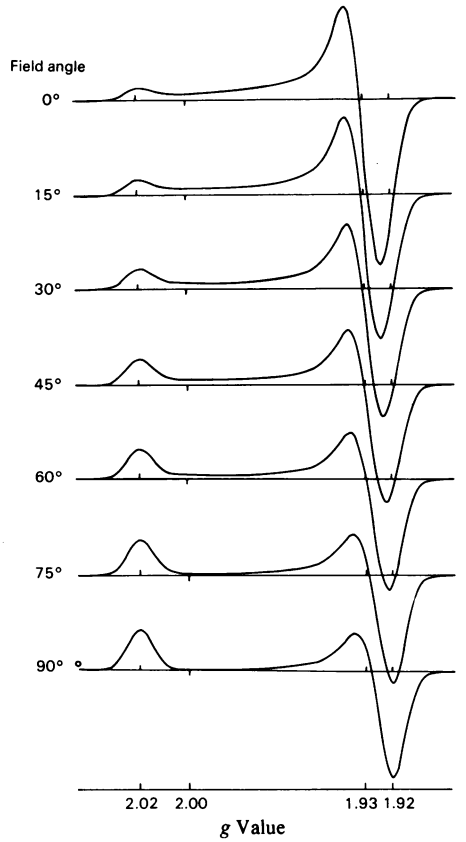

Fig. 2. *Computer simulation of e.p.r. signals from a centre with rhombic symmetry, and their dependence on orientation in the magnetic field*

The principal g axes of the centre are $g_x = 1.92$, $g_y = 1.93$, $g_z = 2.02$, simulating the experimental data of Fig. 1. The linewidths are assumed to be constant at 1 mT and the line shape Gaussian. The mosaic disorder was taken to be 35°. The g_y axis was assumed to lie along the normal to the membrane multilayer and the g_x and g_z axes to be in the multilayer plane. Field angles are as defined in the legend to Fig. 1.

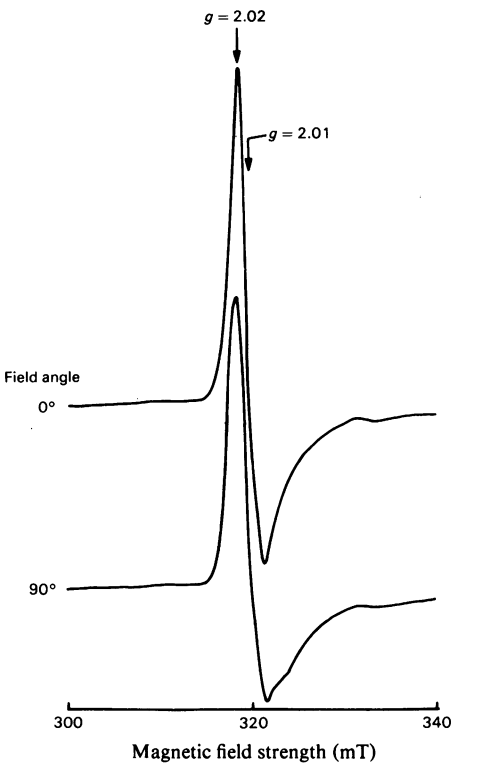

Fig. 3. *Signal from a Hipip seen in membrane multilayers handled anoxically*

Membrane particles were reduced with succinate before centrifugation in deoxygenated, succinate-containing buffer to form multilayers. Slow, partial dehydration was accomplished at 4°C in an anoxic gas phase. Partial oxidation of the multilayers was evident from the signals of high-spin ($g = 6.0$) and low-spin ($g = 2.25$) cytochromes (results not shown). E.p.r. conditions were as described for Fig. 1, except that the temperature was 6.5 K.

45K (results not shown) revealed the $g = 2.02$, $g = 1.93$ and $g = 1.92$ signals seen in fully-reduced multilayers (Fig. 1). The angular dependence of these signals was modest, but clearly equivalent to that of the fully-reduced multilayers. In addition, new signals were seen at $g = 2.09$, $g = 2.04$ and $g = 2.03$. The $g = 2.04$ signal had maximal amplitude at a field angle of 0°, whereas the $g = 2.03$ signal was best seen at 90°. This may arise because the $g = 2.04$ signal obscures the $g = 2.03$ signal least at 90°. The amplitude of the prominent $g = 2.09$ signal was clearly maximal at 90°. Decreasing the sample temperature to 7K resulted in temperature saturation of the $g = 2.09$ signal, but the $g = 2.04$ signal was still clearly seen (results not shown) and again exhibited maximal amplitude at 0°. Thus, two distinct centres contribute to this region of the spectrum: one has its g_z axis in the membrane plane (the $g = 2.09$ signal) whereas the other has its g_z axis lying along the membrane normal ($g = 2.04$). A similar orientation has been deduced for Rieske's centre when the mitochondrial cytochrome bc_1 complex is incorporated into liposomes (Erecińska & Wilson, 1979).

Signals observed in membranes prepared from a cytochrome-deficient mutant

When the mutant strain A1004a is grown on glucose in the absence of δ-aminolaevulinate, it is unable to synthesize haem. However, based on reconstitution experiments with haematin and ATP, Haddock (1973) has concluded that the apoproteins of *b*-type cytochromes are synthesized and incorporated into the membrane of the mutant in the absence of haem synthesis. Little more is known about the composition or arrangement of respiratory chain components in these cytochrome-deficient membranes. However, the finding that such preparations catalyse an ATP-dependent, uncoupler-sensitive reduction of NAD^+ when supplied with glycero-1-phosphate (Poole & Haddock, 1974) suggests that some electron transport components functional in the 'Site-I region' of the respiratory chain are synthesized.

Fig. 4 shows the spectrum of an air-oxidized suspension of (unoriented) membrane vesicles from the mutant at 8K. The most prominent features are at $g = 4.2$, due to 'displaced haem' (Blumberg, 1967) and a Hipip-type signal at $g = 2.02$ and $g = 2.01$. Very high receiver sensitivity is required to see the signals due to residual cytochromes. The high-spin cytochrome signal ($g = 6$) is more readily identifiable than the signals from low-spin cytochromes expected at $g = 3.0$ and $g = 2.25$.

Reduction of the particles in suspension with dithionite (Fig. 5) elicits a spectrum with many features seen in the parental strain, notably signals at $g = 2.09$, 2.02, 2.00 and 1.93. The last signal is small

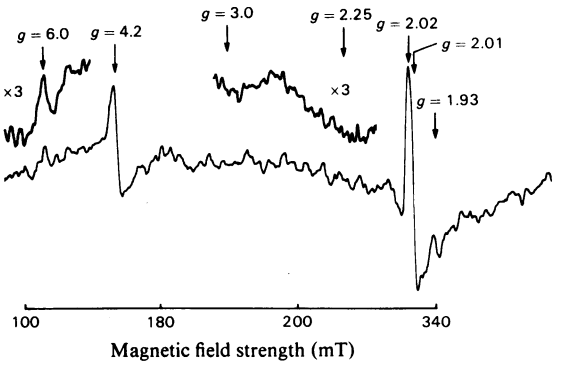

Fig. 4. *E.p.r. spectra of air-oxidized, unoriented membrane particles from a cytochrome-deficient mutant of E. coli*
Experimental conditions were as described for Fig. 1 except that an untreated suspension of membrane particles was used and the temperature was 8 K. The inserts (upper traces) were taken at 3 × the receiver gain used for the complete spectrum (lower trace).

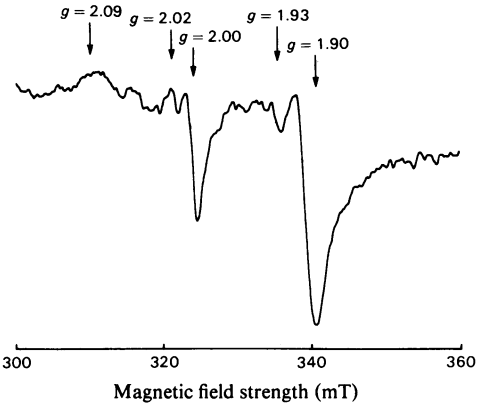

Fig. 5. *E.p.r. spectra of dithionite-reduced, unoriented membrane particles from the cytochrome-deficient mutant*
Experimental conditions were as described for Fig. 1; a solution of $Na_2S_2O_4$ was used as reductant for the suspension of membrane particles. Temperature was 14 K.

in the particles from the mutant. In addition, there is a new major signal at $g = 1.90$. The identity of this signal is unclear. It appears spectrally distinct from a $g = 1.875$ signal seen in the wild-type at low temperatures (< approx. 20K). We have not, however, examined the wild-type strain grown under these conditions (with glucose as carbon source and with only moderate aeration).

To investigate the orientation of the signals, multilayers were prepared as described for particles

from the parental strain. They were reduced by flooding with a solution of $Na_2S_2O_4$. In numerous reduced multilayers, the centre with g values $g = 2.02$, $g = 1.93$, $g = 1.92$ was not well oriented: Fig. 6 shows a typical result. Note that the unidentified $g = 1.90$ signal is clearly oriented, however, exhibiting a maximum amplitude at a field angle of 90° and therefore unlike the $g = 1.90$ signal in mitochondria reported by Erecińska *et al.* (1978). The amplitude decreases monotonically when the multilayer is rotated, to reach a minimum at a field angle of 0°. It is thus oriented in the membrane in a manner analogous to the orientation of Rieske's centre, i.e. the signal is maximal when the magnetic field is parallel to the plane of the multilayer (Salerno *et al.*, 1979). The observed orientation of this signal demonstrates that the multilayer itself is well oriented.

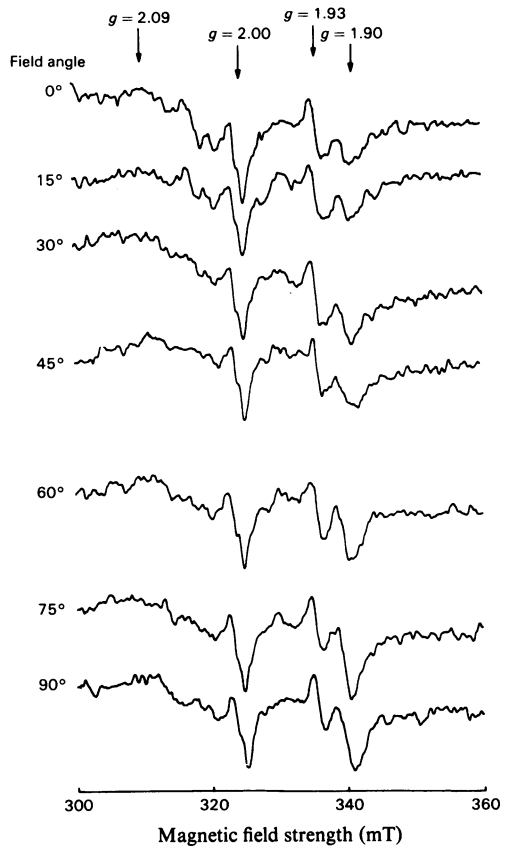

Fig. 6. *Angular dependence of the e.p.r. spectra of dithionite-reduced membrane multilayers from the cytochrome-deficient mutant*

Experimental conditions were as described in the legend to Fig. 1 except that the temperature was 16K.

As prepared, without addition of dithionite or any oxidant, the multilayers from the cytochrome-deficient mutant did not show the Hipip signal. This signal could be demonstrated, however, by flooding the multilayers with a solution of cerium ammonium nitrate, but the signal amplitude was not dependent on the rotation of the multilayer within the magnetic field (results not shown). These cytochrome-deficient particles, therefore, present an intriguing puzzle. Although iron–sulphur clusters that are perhaps comparable with centres S-1 and S-3 in mitochondria are synthesized, they do not (with the exception of the centre with a $g = 1.90$ signal) appear to be ordered in the membrane in any discernible pattern and in this respect differ from their counterparts in the parental strain. We have not yet characterized further the iron–sulphur clusters in the mutant and cannot comment on their roles as electron or hydrogen carriers in the respiratory chain. Based on experiments with a δ-aminolaevulinic acid-requiring mutant of *Bacillus subtilis*, Holmgren *et al.* (1979) have recently suggested that succinate dehydrogenase is synthesized as a soluble protein and becomes membrane bound only when it attaches to a site in the membrane (part of) which is a *b*-type cytochrome. Furthermore, evidence for a structural role for cytochrome *b* in the mammalian respiratory chain comes from the observation of Bruni & Racker (1968) who demonstrated that cytochrome *b* was required for the reconstitution of succinate–coenzyme Q reductase, even though it did not participate in electron transfer from succinate to coenzyme Q.

Conclusions

We have identified several major iron–sulphur clusters in membranes from *E. coli* and described their orientation as far as current information on the molecular geometries of such clusters allows.

The triplet of signals ($g = 1.92$, $g = 1.93$, $g = 2.02$) is tentatively assigned to a low-potential (−220mV) iron–sulphur cluster, analogous to cluster S-2 in succinate dehydrogenase of mitochondria. Orientation studies suggest that its intramembrane organization is also equivalent, the g_y ($g = 1.93$) axis lying at a normal to the membrane plane, and the other two principal g axes lying in the membrane plane. Fig. 7 shows this orientation diagrammatically. A cluster with similar spectral properties appears to have no preferred orientation in the membrane from a cytochrome-deficient mutant; it is possible that in some way the insertion of cytochromes into the membrane provides a 'key' for proper alignment of other respiratory chain components or that the apparently-random orientation in the mutant's membranes reflects a non-functional role for this cluster. Likewise, the sig-

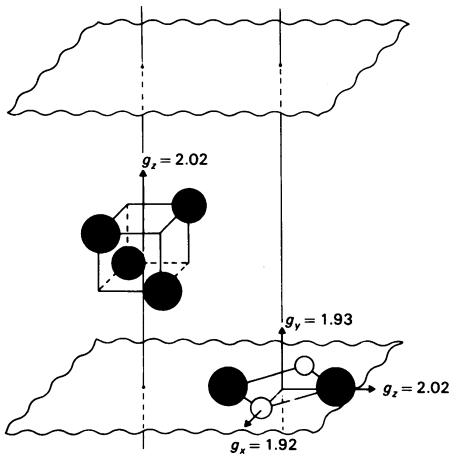

Fig. 7. *Diagram to illustrate the proposed orientation of iron–sulphur clusters in the cytoplasmic membrane of E. coli*

The plane of the membrane is horizontal (almost perpendicular to the page). The upper and lower faces of the membrane are shown. The structure on the left is a tetranuclear cluster (bacterial ferrodoxin and Hipip types) containing four iron atoms (shaded) and four acid-labile sulphur atoms. The $g_z = 2.02$ signal lies parallel to the membrane normal. Since no model exists which relates the magnetic anisotropy to the structure of tetranuclear iron–sulphur clusters, the illustrated orientation of this component is only assumed. The structure on the right is a binuclear cluster (plant ferrodoxin type) containing two iron atoms (shaded) and two acid-labile sulphur atoms. The structure shown is that of Gibson *et al.* (1966) in which the $g_z = 2.02$ axis corresponds to the Fe–Fe axis and is shown as lying in the membrane plane. The $g_x = 1.92$ axis also lies in this plane, whereas the $g_y = 1.93$ axis lies parallel to the membrane normal.

nificance of the high degree of orientation of an unidentified $g = 1.90$ signal in the mutant is unclear at present. The Hipip signal appears analogous to centre S-3 of succinate dehydrogenase. Our data are consistent with the g_z axis ($g = 2.02$) of this tetranuclear structure lying perpendicular to the membrane plane as shown schematically in Fig. 7. Unfortunately, no model exists that relates the magnetic anisotropy to the structure of these iron–sulphur clusters. The results reported here are preliminary in the sense that many of the centres whose orientation is described remain to be explored in more detail in order to determine their functions. However, the finding that the techniques for preparation of oriented multilayers, devised for other membrane particles, are directly applicable to membranes derived from *E. coli* by sonication, opens the way to a detailed study of the molecular organization of membrane-bound redox carriers in *E. coli.*

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