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The orientation of the three haems of the '*in situ*' ubiquinol oxidase, cytochrome *bd*, of *Escherichia coli*

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The *Escherichia coli* cytochrome *bd* complex incorporates three haems as prosthetic groups. In the ferric form these are a predominantly high-spin chlorin (haem *d*), a high-spin haem *b* (b_{595}) and a low-spin haem *b* (b_{558}). The orientations of these three haems have been determined by e.p.r. studies on oriented multilayer preparations of cytoplasmic membrane fragments. The low-spin haem *b* (b_{558}) and the high-spin haem *d* are oriented with their haem planes perpendicular to the membrane plane. The high-spin haem b_{595} is oriented with its haem plane at approx. 55° to the membrane plane. A minor low-spin component, attributable to a low-spin subpopulation of the haem *d*, is also oriented with its haem plane perpendicular to the membrane plane.

INTRODUCTION

Two obiquinol:oxygen oxidoreductases are synthesized by wild-type *Escherichia coli*. When grown under high aeration cytochrome *bo* predominates; when grown under low aeration, a second oxidase, cytochrome *bd* is synthesized as the major ubiquinol oxidase (Rice & Hempfling, 1978; Reid & Ingledew, 1979). Unlike cytochrome *bo* and the mammalian cytochrome aa_3, cytochrome *bd* does not contain copper (Kita *et al.*, 1984; Salerno *et al.*, 1990); it contains three haem components, haem b_{558}, haem b_{595}, and haem *d* (Lorence *et al.*, 1986). The haem *d* has been identified as a chlorin (Timkovich *et al.*, 1985). Although cytochrome *bo* has many similarities, including sequence similarity, to the mammalian enzyme, cytochrome aa_3, no such similarity exists for cytochrome *bd*. Unlike cytochromes aa_3 and *bo*, cytochrome *bd* probably does not function as a proton pump (Miller & Gennis, 1985; Puustinen *et al.*, 1989).

The optical spectrum of the haem b_{558} component of cytochrome *bd* has a characteristic α band at around 560 nm in room temperature reduced-minus-oxidized spectra (Koland *et al.*, 1984): the e.p.r. spectrum of this low-spin ferrihaem is difficult to observe. Only a broad asymmetric g_z feature at $g = 3.3$ has been attributed to haem b_{558} (Meinhardt *et al.*, 1989; Rothery & Ingledew, 1989). The haem b_{595} and *d* components of the enzyme have optical bands at room temperature at 595 nm and 628 nm respectively in reduced-minus-oxidized spectra (Koland *et al.*, 1984). Under aerobic oxidizing conditions these high-spin species have axial or nearly axial e.p.r. spectra with $g_x \sim g_y \sim 6.0$ and $g_z \sim 2.0$. Under anoxically oxidizing conditions the intensity of the axial high-spin species is reduced and a new rhombic high-spin ferric haem species appears with features at $g = 6.3$ (g_x) and 5.6 (g_y); this species has been attributed to haem b_{595} (Meinhardt *et al.*, 1989; Rothery & Ingledew, 1989). In addition to these high-spin ferric haem signals, cytochrome *bd* exhibits a low-spin signal with features at $g = 2.5$ (g_z), 2.3 (g_y), and 1.85 (g_x), which has been attributed to a low-spin subpopulation of haem *d* (Meinhardt *et al.*, 1989). The concentration of this species is small compared with those of the high-spin ferric haems observed by e.p.r. Both haem *d* and haem b_{595} may bind ligands (Poole *et al.*, 1981), and it has been suggested that these two centres interact co-operatively in the oxidase reaction itself (Rothery *et al.*, 1987).

In the present paper we report an e.p.r. study of oriented multilayers of *E. coli* cytoplasmic membranes and we show that the three haem components of cytochrome *bd*, namely haems b_{558}, b_{595}, and *d*, are highly oriented with respect to the membrane plane.

METHODS

Growth of cells and preparation of cytoplasmic membranes

Two strains of *E. coli* were used in the present study: *E. coli* EMG2 (prototroph) strain (N.C.I.B.+10124) and *E. coli* FUN4/pNG2 (Fang *et al.*, 1989). The latter has a point mutation in *cyo* and has the *cyd* operon present on a multicopy plasmid containing the gene for tetracycline resistance. FUN4/pNG2 cells were grown aerobically in batch culture on mineral-salts medium (Cohen & Rickenberg, 1956) with manganese omitted. The medium was supplemented with 0.1 % (w/v) acid-hydrolysed casein and tetracycline (5 mg·l^{-1}). The major reductant and carbon source was glucose (1 %, w/v). Cells were grown to late exponential phase at 37 °C with high aeration. *E. coli* strain EMG2 (prototroph) was grown anaerobically on glycerol with fumarate as respiratory oxidant (Ingledew, 1983). Cells were harvested and cytoplasmic membranes prepared as previously described (Rothery *et al.*, 1987).

Preparation of oriented multilayer e.p.r. samples

Orientated multilayers were prepared using the method of Blum *et al.* (1978*a*,*b*). Cytoplasmic-membrane particles were deposited on acetate sheets by centrifugation in a swinging-bucket rotor for 90 min at 50000 *g* at 4 °C using inserts machined from polycarbonate. The multilayers were partially dried under conditions of approx. 85 % relative humidity at 4 °C for 48–72 h. Samples were cut into strips and inserted into standard quartz e.p.r. tubes (3 mm approximate internal diameter). They were stored frozen under liquid nitrogen. The angle between the plane of the sheet and the applied magnetic field was measured using a pointer fixed to the top of the e.p.r. tubes. An alternative method of oriented multilayer preparation was also used. Samples were produced by centrifugation as described above, but dried quickly (1 h) under a stream of N_2 or CO gas. The gas was dispersed through the coarse-sintered-glass plug of an inverted funnel under which the multilayer sample was fixed to an empty filter paper support from a two-piece polypropylene Buchner funnel (9 cm diameter) that had numerous small (1.5 mm) holes to allow the free passage of gas. This method

produced highly oriented samples as good as those made by slow drying if the samples were removed at the optimum time.

E.p.r. spectroscopy

E.p.r. spectra were recorded using a Bruker ER 200D spectrometer (Bruker Spectrospin) equipped with an Oxford Instruments ESR-9 liquid-helium cryostat and transfer line. Computer simulations of the e.p.r. spectra of oriented multilayers used versions of previously described routines running on the IBM 3081D computer at Rensselaer Polytechnic Institute (Blum *et al.*, 1978*a*,*b*).

RESULTS

The orientations of the three haem planes of cytochrome *bd* (haem b_{558}, b_{595} and *d*) with respect to the cytoplasmic membrane were investigated by using multilayers prepared from membranes of both a prototrophic strain (EMG2) with normal expression levels of cytochrome *bd* and from a strain in which the expression of cytochrome *bd* is amplified and the other oxidase cytochrome *bo* is absent (FUN4/pNG2). The three haems exhibit four e.p.r. spectra; the haem b_{595} gives rise to a high-spin spectrum, the haem *d* to a major high-spin and a minor low-spin and the b_{558} to a low-spin spectrum (Meinhardt *et al.*, 1989; Rothery & Ingledew, 1989). All these were found to be ordered in the multilayers.

Orientation of the haem groups in *E. coli* EMG2

When wild-type *E. coli* is grown anaerobically on glycerol with fumarate as respiratory oxidant, the haem content of its cytoplasmic membranes is dominated by cytochrome *bd* (Reid & Ingledew, 1979; Rothery & Ingledew, 1989). In cytoplasmic membranes from these cells, haem b_{558} is readily detectable optically, but its low-spin e.p.r. spectrum is difficult to observe. A signal which is attributable to haem b_{558} can be observed at $g = 3.3$ (Rothery & Ingledew, 1989). In the present study, this g_z resonance of haem b_{558} was observed, but with difficulty. This signal reaches maximum intensity when the plane of the membrane is within 30° of the magnetic field (results not shown). Owing to the low intensity of the g_z feature in EMG2 membranes, the data quality is poor (therefore we could not determine the angle with greater accuracy than 30°). Nonetheless, these observations are consistent with the more detailed resolution obtained with *E. coli* FUN4/pNG2 (see below).

The high-spin haem spectra of membranes from anaerobically grown EMG2 comprise two components attributable to haems b_{595} and *d*. Under oxically oxidizing conditions both give axial spectra, although the haem b_{595} does have a minor rhombic subspecies (Rothery & Ingledew, 1989; Meinhardt *et al.*, 1989). Spectra taken at different angles of the membrane plane to the magnetic field are shown in Fig. 1. The major component of these spectra is the axial signal at $g = 6.0$ attributable to both haems *d* and b_{595}. On either side of this axial signal, wings attributable to a subspecies of haem b_{595} can be observed. The $g = 6.0$ signal amplitude shows angular dependence (Fig. 2), reaching maximum intensity when the magnetic field is at an angle of approx. 60° to the membrane plane. This is not as marked as that observed in the rhombic component, because two differently oriented species are contributing (see below). The orientation-dependencies of the high-spin signals are similar to those found in *E. coli* FUN4/pNG2 (see below). This orientation-dependence can be simulated as the sum of two components, one oriented with its plane (g_{xy}) 57.5° to the membrane plane and the other oriented with its plane (g_{xy}) perpendicular to the membrane plane in a 4.3:1 ratio. On the basis of the haem stoicheiometry of cytochrome *bd*, it would be expected that this ratio would be close to 1:1; however, there are a number of explanations for the anomalous ratio reported herein (see the Discussion section).

Orientation of the haem groups in *E. coli* FUN4/pNG2

Aerobically grown *E. coli* FUN4/pNG2 has amplified levels of cytochrome *bd*, facilitating resolution of the e.p.r. spectra of its constituent prosthetic groups. Shown in Fig. 3 are e.p.r. spectra

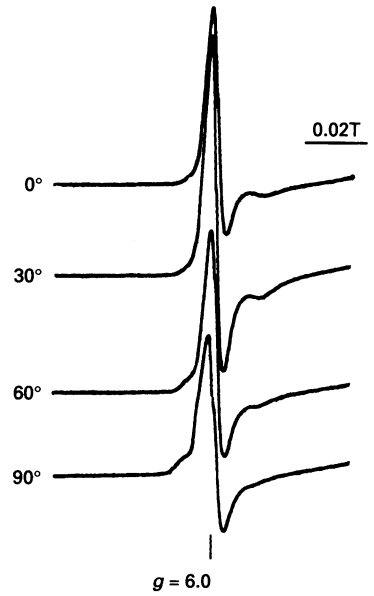

Fig. 1. E.p.r. spectra of the $g = 6.0$ spectral region of oriented multilayers made from *E. coli* EMG2 membranes

Spectra were obtained with the membrane plane 0°, 30°, 60° and 90° to the magnetic field as indicated. E.p.r. spectrometer conditions: temperature, 7 K; modulation amplitude, 1 mT; modulation frequency, 100 kHz; microwave frequency, 9.45 GHz; microwave power, 10 mW; receiver gain, 1×10^5.

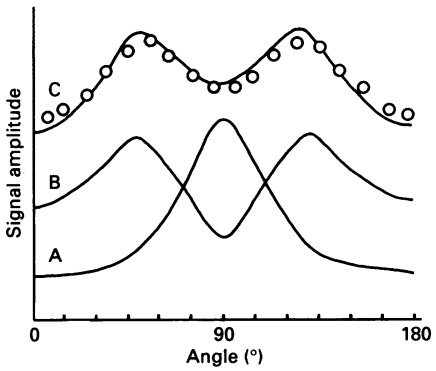

Fig. 2. Plot of the axial $g = 6.0$ signal height as a function of the angle between the magnetic field and the membrane plane in oriented multilayers of *E. coli* EMG2 membranes

Measurements (○) were taken from spectra (peak-to-trough) obtained as described in Fig. 1. The constituent curve peaking at 90° is attributed to haem *d* and that peaking at 57.5° to haem b_{595}. Curve A is a simulation of the angular dependence of peak height for a high-spin haem oriented perpendicular to the membrane plane, assuming 15° disorder in the sample. Curve B is a simulation of the angular dependence of peak height for a high-spin haem oriented with its haem plane at 57.5° from the membrane plane, assuming 15° disorder. Curve C is generated from a sum of curves A and B in a 1:4.3 ratio.

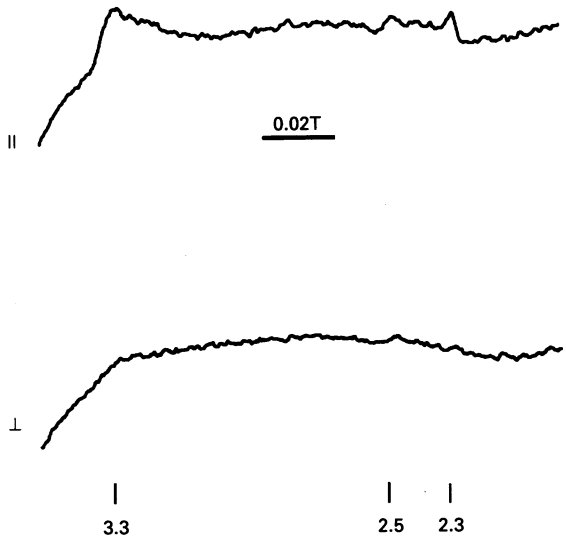

Fig. 3. E.p.r. spectra of oriented multilayers showing the low-spin cytochrome resonances in *E. coli* FUN4/pNG2

The spectra shown were obtained with the membrane plane parallel (top) and perpendicular (bottom) to the magnetic field. E.p.r. conditions: temperature 8 K, modulation amplitude 2 mT, modulation frequency 100 KHz, microwave frequency 9.45 GHz, microwave power 20 mW, receiver gain 2.5×10^4.

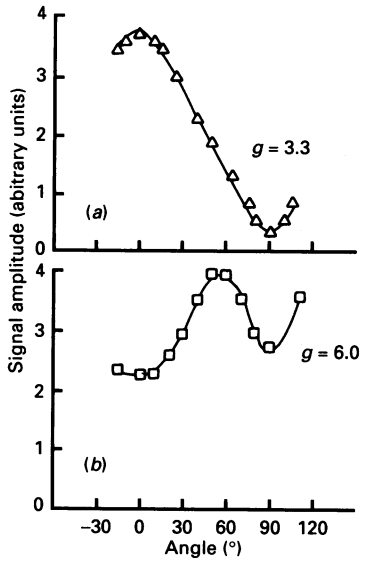

Fig. 4. Signal height of the $g = 3.3$ and $g = 6.0$ signals as a function of angle in oriented multilayers of *E. coli* FUN4/pNG2 membranes

(*a*) Angular dependence of the $g = 3.3$ resonances signal height. Measurements were made from spectra obtained as described in Fig. 3. (*b*) Angular dependence of the axial $g = 6.0$ resonances signal height. Measurements were made from spectra obtained as described in Fig. 5.

of multilayers in the $g = 3$ region; the spectra were taken in the parallel and perpendicular orientations (of the membrane plane to the magnetic field). The g_z peak of haem b_{558} at $g = 3.3$ can be observed. A plot of signal height of this signal as a function of angle is shown in Fig. 4(*a*). This shows that the g_z is parallel with the membrane plane.

In Fig. 3, minor features at $g = 2.5$ (g_z) and 2.3 (g_y) can also be observed; these correspond to a low-spin subspecies of haem

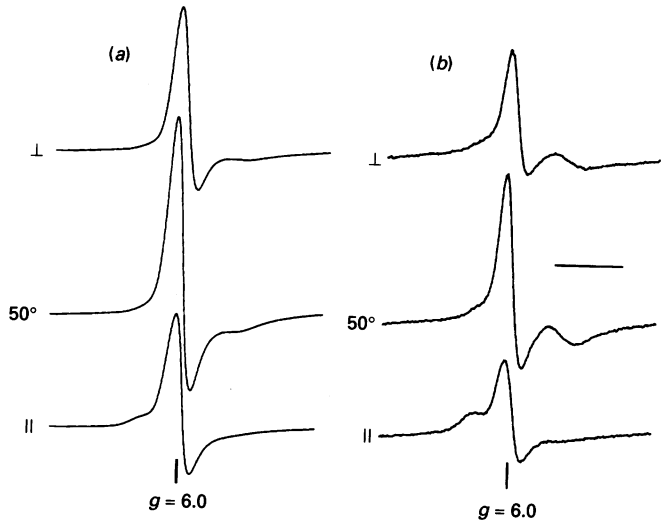

Fig. 5. E.p.r. spectra of the $g = 6.0$ region of oriented multilayers of membranes of *E. coli* FUN4/pNG2 taken at different angles of the membrane plane to the magnetic field

(*a*) Spectra of oxically oxidized samples. The spectra shown were obtained at perpendicular, 50° and parallel orientations. (*b*) Spectra of CO-treated oriented multilayers. The spectra shown were obtained at perpendicular, 50° and parallel orientations. The samples were prepared and treated with CO as described in the text. The bar represents 0.01 T. The e.p.r. conditions were: temperature, 8.5 K; microwave frequency, 9.46 GHz; microwave power, 20 mW; modulation amplitude, 2 mT; modulation frequency, 100 kHz; receiver gain, 5×10^3.

d (Meinhardt *et al.*, 1989). These resonances are better observed at higher temperatures, owing to a slower spin-lattice relaxation rate than for the other haem components (results not shown). Computer fitting of the orientation-dependence of the low-spin haem *d* signals shows that both the g_z and g_y tensors lie within 15° of the membrane plane (not shown).

The high-spin ferric haem e.p.r. signals of *E. coli* FUN4/pNG2 membranes are similar in lineshape and orientation-dependence to those of *E. coli* EMG2. Spectra are shown in Fig. 5(*a*); the angular dependence of the signal height of the $g = 6.0$ signal is plotted in Fig. 4(*b*). The signal is maximal when the magnetic field is about 55° from the plane of the membrane. As in the case of *E. coli* EMG2, this is a composite angular dependence which needs to be deconvoluted. Furthermore, the signal arises from all the in-haem-plane orientations of the magnetic field, and hence less information is available than if it were due to a unique orientation in the *g*-tensor co-ordinate system.

To extract the orientations of the two high-spin components, we sought additional information. We decided to allow one of the haem groups to form a complex with an exogenous ligand. A compound of the cytochrome *bd* complex in which haem b_{595} is oxidized and *d* is reduced and liganded to CO can be readily formed: the 'mixed-valence-CO' compound (Rothery & Ingledew, 1989). In this compound the spectrum of the b_{595} becomes rhombic. It did not prove possible to convert multilayers made by the usual method into this redox state. Multilayers were made by centrifugation of *E. coli* FUN4/pNG2 cytoplasmic membranes in CO-saturated buffer. The membranes, deposited on the acetate discs, were dried under a stream of CO gas as described in the Methods section. Fig. 5(*b*) shows e.p.r. spectra of multilayers prepared in this way. The axial signal is relatively smaller, and a strong rhombic signal is observed. In these samples the 'mixed-valence-CO' compound has not fully formed, as indicated by the residual axial species. The orientation of the axial $g = 6.0$

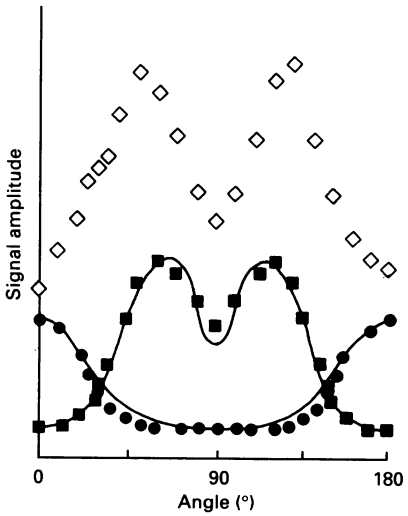

Fig. 6. Signal height of the high-spin resonances in CO-treated oriented multilayers of membranes from *E. coli* FUN4/pNG2 plotted as a function of angle

Measurements were taken from spectra obtained as in Fig. 5(*b*). The $g = 6.0$ (◇), $g = 6.3$ (●), and $g = 5.6$ (■) signal heights are plotted against the angle between the membrane plane and the magnetic field. The curves associated with the $g = 6.3$ and $g = 5.6$ signal heights are simulations based on a linewidth of 3 mT, a disorder of 20° and the $g = 6.3$ direction being in plane and the $g = 5.6$ direction being at 57.5° from plane.

signal is similar to that of the oxically oxidized samples, but its relative intensity is decreased. The peak at $g_x = 6.3$ is most intense with the field in the plane of the membrane, whereas the $g_y = 5.6$ feature reaches its maximum at approx. 55° from the membrane plane, as compared with approx. 50° for the maximum of the $g_{xy} = 6.0$ feature in this case (Fig. 6). The angular dependence of the rhombic high-spin signals can be simulated with the assumption that they arise from a single ferrihaem group oriented so that the haem plane is 57.5° from the plane of the membrane. The axis of the *g*-tensor coordinate system corresponding to $g_x \sim 6.3$ is within 10° of the membrane plane. The other in (haem)-plane axis, corresponding to $g_y \sim 5.6$, is oriented at 57.5° to the membrane plane. The disorder parameter used in the simulations is 20°. These findings on the CO-treated membranes are qualitatively the same as with the minor rhombic high-spin species observed in oxically prepared samples of both EMG2 and FUN/pNG2.

DISCUSSION

The orientation of the three haem groups is the same in both the wild-type and over-expressing strains. The low-spin haem b_{558} is oriented with its plane perpendicular to the plane of the membrane. This is the orientation of haem b_{562} and the haem b_{556} of the mitochondrial cytochrome bc_1 complex, of haem *a* and a_3 of mitochondrial cytochrome aa_3, of the chloroplast cytochrome b_{559} (Blum *et al.*, 1978*a*,*b*), and of the two haem groups of cytochrome *o* from *E. coli* (Salerno & Ingledew, 1991). The orientation is one that may be expected of a haem group suspended between transmembrane sections of α-helix, and it is possible that the haem is located between two such helices in the bilayer-spanning region of the complex.

The determination of angular dependence of the high-spin haem e.p.r. signals is complicated by two factors. In the air-oxidized complex, both haem b_{595} and haem *d* contribute nearly axial e.p.r. spectra with $g_x \sim g_y \sim 6.0$ and $g_z \sim 1.99$, and the observed orientation-dependence of these features is the sum of the orientation-dependencies of the two high-spin haem components. Also, because these species are nearly axial, the $g = 6$ feature of each does not correspond to a unique orientation. Magnetic-field orientation along any direction in the *x*–*y* plane of the *g*-tensor co-ordinate system will contribute to the $g = 6.0$ signal.

The data are further complicated by the ill-defined redox state of the cytochrome *bd* complex in membrane multilayers. The air-oxidized complex contains oxygen ligated to ferrous haem *d* (Poole *et al.*, 1983). This species, haem d_{650}, is e.p.r.-silent, and its presence in the multilayer samples studies herein probably accounts for the anomalous 4.3:1 ratio reported above for the relative concentrations of the two axial high-spin haem groups. In addition the haem *d*, having the highest potential in the complex, may be partially reduced in the multilayers; further, this ratio may also reflect differences in linewidth or unresolved anisotropy not obvious from the overlapped spectra.

Additional information concerning the orientation of the high-spin haem groups was obtained by drying the multilayers in a stream of CO gas. This produces multilayers in which about half the complexes are one-electron-reduced CO adducts, with the CO bound to ferrohaem *d*. Under these conditions, haem b_{595} is a rhombic high-spin haem with $g_x \sim 6.3$ and $g_y \sim 5.6$. The e.p.r. spectra of the resulting multilayers, shown in Fig. 5, have a large $g_x \sim 6.3$ feature when the magnetic field is in the plane of the membrane; the $g_y \sim 5.6$ feature is largest at intermediate orientation. Plots of signal amplitude against angle from the plane show maxima at 0° and 55° respectively for the $g \sim 6.3$ and 5.6 features, as shown in Fig. 6. Since these features correspond to field orientations in the haem plane and 90° apart, the haem plane of b_{595} must be at 55° from the membrane plane. If we assume that the orientation of this haem group does not change greatly with the redox state of the complex, we can calculate the contribution of an axial haem group with this orientation to the observed orientation-dependence of the $g = 6.0$ signal of oxidized membranes. A reasonable simulation can be constructed with one haem plane oriented at approx. 55° and the other at 90° to the membrane plane (Fig. 2). The summation of these two species gives the fit to the data points shown. The fit to the 55° component is similar to that for g_y of the rhombic component.

It is instructive to consider axial high-spin haem groups of several orientations. A high-spin haem group with its plane perpendicular to the membrane plane would have intensity at $g = 6.0$ at all field orientations relative to the membrane. The largest amplitude would result when the magnetic field was along the normal to the membrane, since all the haem molecules would, neglecting disorder, have $g_x = g_y = 6.0$ along the field direction. By contrast, when the field is in the membrane plane, all values between 6.0 and 2.0 will be presented. A high-spin haem group with its plane parallel with the membrane plane will give rise to a significant $g = 6.0$ signal only when the magnetic field is in, or near, the plane of the membrane; the rate of decrease in signal with angle is determined by the disorder of the sample. When the haem plane is at some intermediate angle (θ) to the membrane plane, only disorder allows any signal amplitude at $g = 6.0$ for field-orientation angles greater than θ. By analogy with the perpendicular case, it might be assumed that the maximum signal amplitude would be observed for field angles near θ; this assumption is not valid. If $\theta = 60°$, *g* values from 3.46 to 6 can be expected (neglecting disorder) for a field orientation angle of 60°, and the spread of *g* values will be the same at a field orientation of 0°. For $\theta = 45°$, the spread of *g* values for a field orientation of 45° is 2.0 to 6.0; maximum intensity can be expected with the field in the membrane plane. For θ less than 45°, a greater spread of *g* values will always be observed for field

orientations along θ than for field orientations in the plane of the membrane. This is one of a number of situations in which the peak of an angular-dependence plot does not necessarily correspond to the orientation of a chromophore *g*-tensor axis.

It is suggested that both the haem *d* and the haem b_{558} are located between two membrane-spanning α-helices and, from a consideration of the amino acid sequence (Green *et al.*, 1988), it is probable that the same helices are responsible for the endogenous ligation of both haem groups. The orientation of haem b_{595} is clearly not consistent with the same structural motif. It may therefore be located outside the membrane bilayer, with the endogenous ligand being supplied by a section of polypeptide which connects two such helical segments, or by a terminal section outside the bilayer. To construct a preliminary model of cytochrome *bd* it is instructive to compare the haem orientation data with what is known about the sidedness of the haem components with respect to the cytoplasmic membrane. The haem b_{558} ubiquinol-oxidizing subunit (subunit I; Green *et al.*, 1984) is located towards the outside of right-side-out proteoliposomes containing cytochrome *bd* (Miller & Gennis, 1985; Lorence *et al.*, 1988). The sidedness of the ligand-binding components of the cytochrome have been investigated in inside-out membrane vesicles and in whole cells by using the effect of the membrane-impermeant paramagnetic probe dysprosium III EDTA on the e.p.r. microwave power saturation of the nitrosyl haem adducts to haem *d* and haem b_{595} (Rothery & Ingledew, 1988). In these studies, the nitrosyl haem derivatives were found to be located approx. 1 nm (10 Å) below the inner surface on the cytoplasmic membrane. It is useful to compare this structural picture with what has been deduced for the cytochrome aa_3 and cytochrome *bo* enzymes. In each of the three oxidases, the low-spin haem has the same orientation. The catalytic sites of the cytochrome aa_3 and cytochrome *bo* systems are probably haem–copper pairs. Whereas the cytochrome aa_3 and cytochrome *bo*, which share extensive sequence similarity, probably have active sites located deep within the bilayer-spanning region, the catalytic site of the cytochrome *bd* system is probably located at the cytoplasmic edge of this region. This may be related to the ability of the cytochrome aa_3 and *bo* systems to pump protons (Puustinen *et al.*, 1989). Cytochrome *bd* lacks a metal centre analogous to the Cu_a of cytochrome aa_3; the former is a quinol oxidase, whereas the latter is a cytochrome *c* oxidase. The binding site for quinol is likely to be partially within the bilayer-spinning region. It may be a slot between adjacent α-helices into which the quinol can insert much as the haem group does, although inhibitor-resistant mutants in cytochrome bc_1 complexes map into hydrophilic regions adjacent to the bilayer (Crofts *et al.*, 1989). It will in any case be much more deeply buried within the membrane than a binding site for a water-soluble globular protein such as cytochrome *c*. The bound semiquinone produced by quinol oxidation represents an additional available reducing equivalent, which may also be significant in dispensing with Cu_a.

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REFERENCES

Blum, H., Harmon, H. J., Leigh, J. S., Salerno, J. C. & Chance, B. (1978*a*) Biochim. Biophys. Acta **502**, 1–10
Blum, H., Salerno, J. C. & Leigh, J. S. (1978*b*) J. Magn. Reson **30**, 385–391
Cohen, G. N. & Rickenberg, H. W. (1956) Ann. Inst. Pasteur **91**, 693–720
Crofts, A. R., Robinson, H., Andrews, K., Van Doer, S. & Barry, E. (1989) in Cytochrome Systems: Molecular Biology and Bioenergetics (Papa, S., ed.), pp. 617–631, Plenum Press, New York
Fang, H., Lin, R.-J. & Gennis, R. B. (1989) J. Biol. Chem. **264**, 8026–8032
Green, G. N., Kranz, R. G., Lorence, R. M. & Gennis, R. B. (1984) J. Biol. Chem. **259**, 7994–7999
Green, G. N., Fang, H., Lin, R., Newton, G., Mather, M., Georgiou, C. D. & Gennis, R. B. (1988) J. Biol. Chem. **263**, 13138–13142
Ingledew, W. J. (1983) J. Gen. Microbiol. **127**, 1651–1659
Kita, K., Konishi, K. & Anraku, Y. (1984) J. Biol. Chem. **259**, 3375–3381
Koland, J. G., Miller, M. J. & Gennis, R. B. (1984) Biochemistry **23**, 1051–1056
Lorence, R. M., Koland, J. G. & Gennis, R. B. (1986) Biochemistry **25**, 2314–2321
Lorence, R. M., Carter, K., Gennis, R. B., Matsushita, K. & Kaback, H. R. (1988) J. Biol. Chem. **263**, 5271–5276
Meinhardt, S. W., Gennis, R. B. & Ohnishi, T. (1989) Biochim. Biophys. Acta **975**, 175–184
Miller, M. J. & Gennis, R. B. (1985) J. Biol. Chem. **260**, 14003–14008
Poole, R. K., Scott, R. I. & Chance, B. (1981) J. Gen. Microbiol. **125**, 431–438
Poole, R. K., Kumar, C., Salmon, I. & Chance, B. (1983) J. Gen. Microbiol. **129**, 1335–1344
Puustinen, A., Finel, M., Virkki, M. & Wikstrom, M. (1989) FEBS Lett. **194**, 163–167
Reid, G. A. & Ingledew, W. J. (1979) Biochem. J. **182**, 465–472
Rice, C. W. & Hempfling, W. P. (1978) J. Bacteriol. **134**, 115–124
Rothery, R. A. & Ingledew, W. J. (1988) Eur. Bioenerg. Conf. Short Rep. **5**, 98
Rothery, R. A. & Ingledew, W. J. (1989) Biochem. J. **261**, 437–443
Rothery, R. A., Houston, A. M. & Ingledew, W. J. (1987) J. Gen. Microbiol. **133**, 3247–3255
Salerno, J. C. & Ingledew, W. J. (1991) Eur. J. Biochem. **198**, 789–792
Salerno, J. C., Bolgiano, B., Poole, R. K., Gennis, R. B. & Ingledew, W. J. (1990) J. Biol. Chem. **265**, 4364–4368
Timkovich, R., Cork, M. S., Gennis, R. B. & Johnson, P. Y. (1985) J. Am. Chem. Soc. **107**, 6069–6075