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Characterization and Phenotypic Control of the Cytochrome Content of *Escherichia coli*

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1. Electron-transport particles derived from *Escherichia coli* grown aerobically contain three *b*-type cytochromes with mid-point oxidation–reduction potentials at pH 7 of +260mV, +80mV and −50mV, with $n = 1$ for each. The variation of these values with pH was determined. 2. *E. coli* develops a different set of *b*-type cytochromes when grown anaerobically on glycerol with fumarate or nitrate as terminal electron acceptor. Electron-transport particles of fumarate-grown cells contain *b*-type cytochromes with mid-point potentials at pH 7 of +140mV and +250mV ($n = 1$). These two cytochromes are also present in cells grown with nitrate as terminal acceptor, where an additional cytochrome *b* with a mid-point potential of +10mV ($n = 1$) is developed. 3. The wavelengths of the α-absorption-band maxima of the *b*-type cytochromes at 77K were: (*a*) for aerobically grown cells, cytochrome *b* (E_{m7} +260mV), 556nm and 563nm, cytochrome *b* (E_{m7} +80mV), 556nm and cytochrome *b* (E_{m7} −50mV), 558nm; (*b*) for anaerobically grown cells, cytochrome *b* (E_{m7} +250mV), 558nm, cytochrome *b* (E_{m7} +40mV), 555nm and cytochrome *b* (E_{m7} +10mV), 556nm. 4. Cytochrome *d* was found to have a mid-point potential at pH 7 of +280mV ($n = 1$). 5. Cytochrome a_1 was resolved as two components of equal magnitude with mid-point potentials of +260mV and +160mV ($n = 1$). 6. Redox titrations performed in the presence of CO showed that one of the *b*-type cytochromes in the aerobically grown cultures was reduced, even at the upper limits of our range of electrode potentials (above +400mV). Cytochrome *d* was also not oxidizable in the presence of CO. Neither of the cytochromes a_1 was affected by the presence of CO.

Escherichia coli contains cytochromes of types *b*, *a*, *d* and *c* (Shipp, 1972). Cytochrome *c* in *E. coli* is reported to be soluble (Fujita, 1966) and is not required for respiratory electron transport. We have investigated the cytochromes *b*, a_1 and *d* of membranes derived from *E. coli* grown under various defined conditions.

Shipp (1972) suggested from fourth-order finite-difference analysis of spectra that aerobically grown cells contain three *b* cytochromes. Hendler *et al.* (1975) resolved three *b* cytochromes by redox potentiometry of membranes derived from aerobically grown *E. coli*, and these had E_{m7} values of +220mV, +110mV and −50mV. Pudek & Bragg (1976) resolved only two major *b* cytochromes with E_{m7} values of +165mV and +35mV in these aerobically grown cells. We have performed redox titrations with aerobically grown *E. coli*, and our data are similar to those obtained by Hendler *et al.* (1975). Possible reasons for the discrepancies in these reported results are discussed.

We have measured the mid-point potentials of cytochromes a_1 and *d*. These cytochromes are not found in vigorously aerated early-exponential-phase cultures, but apparently increase in concentration as O_2 availability decreases. These cytochromes are found in their highest concentrations in the membranes of anaerobically grown cells. *E. coli* possesses an inducible respiratory nitrate reductase (EC 1.7.99.4) and fumarate reductase (EC 1.3.99.1), which allow this organism to grow anaerobically with a non-fermentable carbon source in the presence of nitrate and fumarate respectively. We have also investigated the *b*-cytochrome content of these anaerobically grown cells and find them to be thermodynamically and spectroscopically distinct from the *b* cytochromes of aerobically grown cells.

By determination of photochemical action spectra, Castor & Chance (1959) demonstrated that both cytochromes *o* and *d* can function as cytochrome oxidases in *E. coli* grown aerobically. Although it has been suggested (e.g. Revsin & Brodie, 1969) that cytochrome a_1 is also capable of oxidase activity, this pigment is not kinetically competent to support

Abbreviations used: Mes, 4-morpholine-ethanesulphonic acid; Tes, 2-{[2-hydroxy-1,1-bis(hydroxymethyl)-ethyl]amino}ethansulphonic acid; E_{m7}, mid-point potential at pH 7; E_h, electrode potential relative to the standard hydrogen electrode.

respiration (Haddock *et al.*, 1976). Since it appears that CO, similarly to O_2, binds predominantly to ferrous cytochromes, its presence shifts the apparent mid-point potentials of those cytochromes to which it binds to more positive values. We have performed redox titrations in the presence of CO in order to identify more accurately the likely cytochrome oxidases present under different growth conditions.

We have extended the potentiometric method to obtain low-temperature (77K) spectra of each individual cytochrome by scanning the absorbance difference between samples poised at appropriate electrode potentials.

Materials and Methods

Growth of cells

E. coli strain EMG-2 (prototroph) was grown at 37°C in batches of 20 litres on the mineral salts medium described by Cohen & Rickenberg (1956), supplemented with vitamin-free casamino acids (0.1%, w/v) and either glycerol (0.5%, w/v) or succinic acid (0.5%, w/v). Aerobic growth was effected by sparging the growth medium vigorously with sterile air. Anaerobic growth on fumarate (50mM) or nitrate (100mM) as terminal electron acceptor was carried out in the above medium with glycerol as carbon source after bubbling N_2 gas into the growth vessel before inoculation. An inoculum of 450ml was used for each 20-litre batch, and this was grown aerobically or anaerobically as appropriate.

Harvesting and breakage

Cells were harvested in late exponential phase in an MSE continuous-action rotor (43118-503) at 16000rev./min with a flow rate of approx. 300ml/min, washed with 0.1M-potassium phosphate, pH7.5, and centrifuged at 10000*g* for 15min at 4°C. This washing and centrifugation was repeated once, and the cells were then broken by two passages through the French pressure cell at approx. 120MPa. Electron-transport particles were prepared by the following procedure. Unbroken cells were removed by centrifugation at 10000*g* for 15min at 4°C, the supernatant was decanted off and centrifuged at 100000*g* at 4°C for 1h to pellet the particles. Where stated the electron-transport particles were also washed with 1mM-Tris/HCl, pH7.5, to remove one of the *b*-type cytochromes as described below.

Redox potentiometry

Oxidation–reduction mid-point potentials of the cytochromes were determined at room temperature essentially as described by Dutton (1971). Rather than measuring a single difference by dual-wavelength spectrometry at fixed wavelengths, a spectrum was scanned at each measured electrode potential. By using the single-beam spectrophotometer described below, the sample was oxidized to above +400mV and a spectrum was scanned. At more reduced potentials spectra were similarly scanned, and from these, the initial 'oxidized' spectrum was automatically subtracted. Anaerobiosis was maintained by flushing the cuvette with argon gas.

The following mediators were used: 1,2-naphthoquinone (E_0' +143mV), menadione (+9mV), 2-hydroxy-1,4-naphthoquinone (−145mV), 1,2-naphthoquinone-4-sulphonate (+215mV), duroquinone (+10mV), quinhydrone (+280mV), *N*-methylphenazonium methosulphate (+80mV), trimethylhydroquinone (+240mV). The dye concentrations were varied in the range 10–50μM, depending on the protein concentration in the cuvette. More dye was added if mediation appeared to be inadequate. Small portions of 100mM-ascorbic acid or 100mM-sodium dithionite were used as reductants, and potassium ferricyanide was used as oxidant.

Single-beam spectrophotometer for scanning spectra

This instrument was designed by Professor P. B. Garland of this department. Briefly, it used a 55W quartz/iodide tungsten-filament lamp as the light-source, an $f=6.8$ grating monochromator (type EUE-700, Heath Co., Benton Harbor, MI, U.S.A.) with a stepping-motor wavelength drive, a single 1cm-light-path cuvette positioned over a magnetic stirrer (Rank Brothers, Bottisham, Cambs., U.K.), which rotated a 7mm-long stir bar (Nalgene, model 6610, from Techmate, Luton, Beds., U.K.), and, closely applied to the cuvette, an end-window photomultiplier tube with an S20 response for improved performance in the red (model 9798B; EMI Electronics Tube Division, Hayes, Middx., U.K.). The high-voltage supply for the photomultiplier was a model 472R from Brandenburg Ltd., Thornton Heath, Surrey, U.K. The photomultiplier anode current was taken directly to the virtual earth input of an operational amplifier set up as a logarithmic current-to-voltage converter by using a logarithmic module yielding 1.0V change of output for a tenfold change of current input (model 751N, Analogue Devices, Molesey, Surrey, U.K.). A constant reference current of 0.2μA was also logarithmically amplified by a second operational amplifier using the other channel of the logarithmic module. Changes in the differences between the outputs of these two logarithmic amplifiers give, at constant wavelength, the change in absorbance of the solution being measured. The photomultiplier signal giving 0.2μA anode current was arbitrarily equated with zero absorbance; the sensitivity was 1.0V per absorbance unit. The difference between the logarithmically amplified photomultiplier current and constant reference current was displayed on a centre zero meter,

and was stored during wavelength scanning in a two-channel transient recorder of 10-bit resolution on the input signals (Physical Data Inc., model 514A, Bryans Southern Ltd., Mitcham, Surrey, U.K.).

In practice, the photomultiplier supply was adjusted at the start of a titration to give an approximately zero reading on the display meter, and the capacity of the transient recorder used to accommodate varying signal levels as the wavelength changed during scanning. The wavelength scan for a redox titration was usually about 100nm or even less, covering either the Soret region or the α-band region. Longer scans are less useful because they run into absorbance changes of the mediators. Within these relatively limited wavelength scans, no difficulty was experienced in choosing a starting value for the photomultiplier high voltage such that all signals remained within the limits of the input range of the transient recorder, usually ± 0.2 or ± 0.5V (i.e. ± 0.2 or ± 0.5 absorbance change respectively). Any one wavelength scan placed into the memory represented an absolute spectrum that was uncorrected for instrumental factors such as the wavelength-dependence of the light-source output, monochromator throughput and photomultiplier sensitivity. However, the differences between two such stored spectra (gathered over the same photomultiplier gain and wavelength range) are true difference spectra, corrected for instrumental factors. Thus by initially placing the uncorrected spectrum from an initial scan at the starting redox potential into the first channel of the transient recorder, and keeping it there, subsequent spectra measured on the preparation at different potentials could be placed into the second channel and corrected by subtraction of the first channel ('baseline') before presentation on an oscilloscope or pen recorder as true difference spectra.

Low-temperature spectroscopy

Difference spectra at 77K were obtained by using a split-beam scanning spectrophotometer (Haddock & Garland, 1971). For reduced-minus-oxidized spectra, a cuvette with 2mm light-path was used. Samples poised at appropriate electrode potentials were collected in 3mm-internal-diameter quartz tubes and rapidly frozen to 77K. Difference spectra between any desired pair of samples were then scanned.

Materials

Vitamin-free casamino acids were obtained from Difco (Detroit, MI, U.S.A.), argon (<1p.p.m. of O_2) was from Air Products (Crewe, Cheshire, U.K.) and CO (<2p.p.m. of O_2) from British Oxygen (London, U.K.). 1,2-Naphthoquinone and 1,2-naphthoquinone-4-sulphonate were obtained from Koch–Light Laboratories (Colnbrook, Bucks., U.K.), 2-hydroxy-1,4-naphthoquinone and duroquinone from Ralph Emanuel (Wembley, Middx., U.K.), and trimethylhydroquinone was from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). All other reagents were obtained from BDH Chemicals (Poole, Dorset, U.K.) and were of the highest available purity.

Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin (Sigma; fraction V) as standard.

Results and Discussion

Typical spectra of the preparations used in our experiments are shown in Fig. 1. That the cytochrome content of these preparations varies with growth conditions is clear. The nature of these differences is investigated below. It is noteworthy that our aerobically grown cultures contained little cytochrome a_1 and d and that cytochrome b_{558} was not detectable (Fig. 1*a*) in these cells.

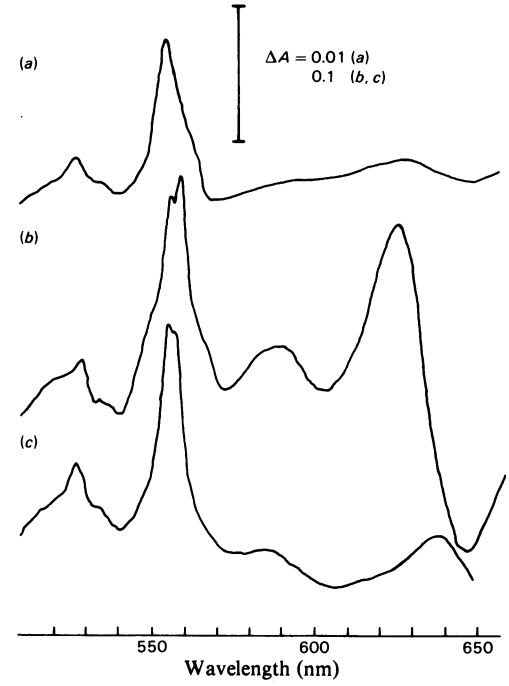

Fig. 1. *Absorbance spectra of membranes from E. coli grown with different respiratory electron acceptors*
Electron-transport particles were prepared from cells grown under different conditions as described in the Materials and Methods section. Samples were reduced with a few grains of sodium dithionite; reference samples were oxidized with H_2O_2. Spectra were scanned at 77K. The membranes were prepared from cells grown (*a*) aerobically with glycerol (11.5mg of protein/ml), (*b*) anaerobically with glycerol and fumarate (7.5mg of protein/ml) and (*c*) anaerobically with glycerol and nitrate (5.4mg of protein/ml).

Aerobically grown cells

The results of a typical redox titration of the *b* cytochromes is shown in Fig. 2(*a*). Two cytochromes *b* are found here, with E_{m7} values of +260mV and +80mV. The pH-dependence of these values and that of a low-potential cytochrome *b* (see below) is shown in Fig. 3. Assuming a dependence of E_m on pH of −60mV/pH unit, cytochromes *b* with E_{m7} values of +260, +80 and −50mV have p*K* values of approx. 7.5, 6.5 and 7.0 respectively. No differences were found between cells grown with glycerol or with succinate as carbon source. The electron-transport particles (Fig. 2) were washed once with 1mM-Tris/HCl, pH7.5. This procedure was found to remove one of the three *b* cytochromes present in the original membrane preparation. This component was found to have a mid-point potential of −50mV at pH7.0 (result not shown). Particles lacking this cytochrome exhibit unimpaired NADH oxidase activity, and although succinate oxidation is decreased, this is probably due to loss of succinate dehydrogenase activity (Table 1). The soluble lower-potential cytochrome *b* is therefore considered not to be a component of the main respiratory pathways.

Redox titrations performed under an atmosphere of CO (Fig. 2*b*) shows only one *b* cytochrome titrating in the normal redox potential range, the other not being oxidized by ferricyanide. Such behaviour is indicative of an oxidase, since such enzymes appear to bind CO when in the reduced (ferrous) state. This binding thus increases the apparent E_{m7} of the cytochrome in question. We find that the single component that titrates in the range accessible to our experimentation has an E_{m7} value of +200mV, which does not correspond directly to either of the values found in the absence of CO. Fig. 2(*a*) shows that approximately twice as much absorbance change can be attributed to the cytochrome *b* titrating at +80mV compared with that at +260mV. Approximately one-third of the total absorbance change found during the titration in the absence of CO is found when CO is present (Fig. 3). This indicates that the high-potential *b* cytochrome does not bind CO, whereas the component titrating at +80mV in the absence of CO does.

By poising samples of electron-transport particles at various electrode potentials we have measured reduced-minus-oxidized difference spectra of the individual components (Fig. 4). The cytochrome *b*

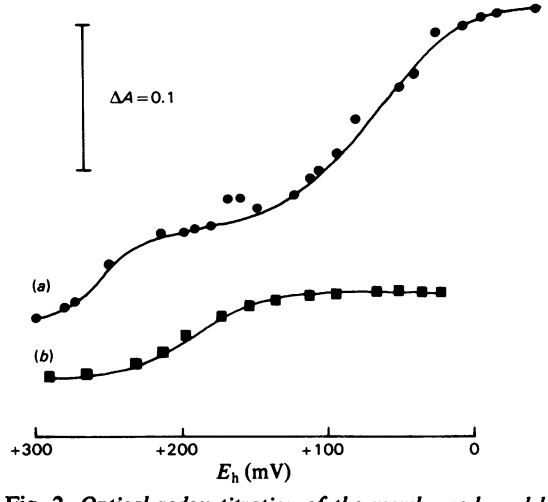

Fig. 2. *Optical redox titration of the membrane-bound b cytochromes from aerobically grown E. coli in the presence and absence of CO*

Redox titrations were performed as described in the Materials and Methods section with membrane particles derived from cells grown aerobically with glycerol, washed with 1mM-Tris/HCl, pH7.5, and resuspended in 50mM-Tes/75mM-KCl, pH7.0. The cuvette was flushed with (*a*) argon and (*b*) CO. The change in absorbance was measured at 559nm. The solid lines are fitted for $n = 1$ components.

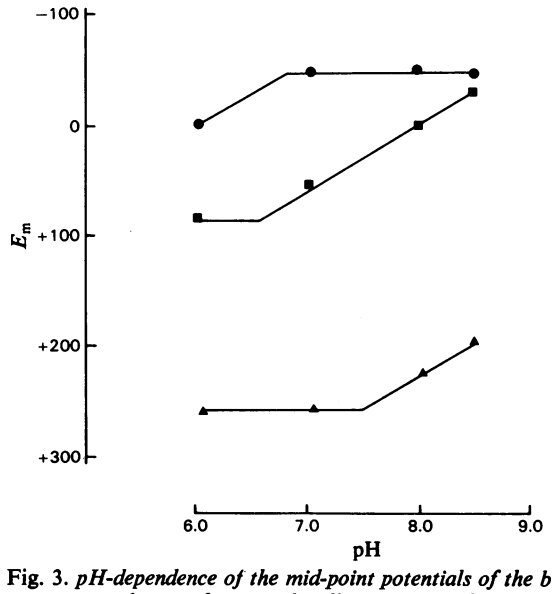

Fig. 3. *pH-dependence of the mid-point potentials of the b cytochromes from aerobically grown E. coli*

Optical redox titrations were performed as described in the Materials and Methods section, with membrane particles derived from cells grown aerobically with glycerol. The particles were suspended in 50mM-Mes/75mM-KCl, pH6.0, 50mM-Tes/75mM-KCl, pH7.0, 50mM-Tris/75mM-KCl, pH8.0, or 50mM-Tris/75mM-KCl, pH8.5. The mid-point potentials of the high-(▲), intermediate-(■) and low-(●) potential *b* cytochromes are plotted as a function of pH.

Table 1. *Oxidase activities of electron-transport particles from aerobically grown E. coli*

Electron-transport particles prepared as described in the Materials and Methods section were suspended in one of the buffers indicated (approx. 1 mg of protein/ml) and centrifuged at 100000*g* for 1 h at 4°C. The pellets were resuspended in 50mM-Tes/75mM-KCl, pH7.0. O_2 uptake was measured in a 1 ml jacketed reaction vessel at 30°C with 1 mM-NADH or 10mM-sodium succinate. Carbonyl cyanide 4-trifluoromethoxyphenylhydrazone (FCCP) was added in each case to a final concentration of 1 μg/ml.

Final washing	NADH oxidase (ng-atoms of O/min per mg of protein)	Succinate oxidase (ng-atoms of O/min per mg of protein)
50mM-Tes/75mM-KCl, pH7.0	400	38
1mM-Tris/HCl, pH7.5	375	23

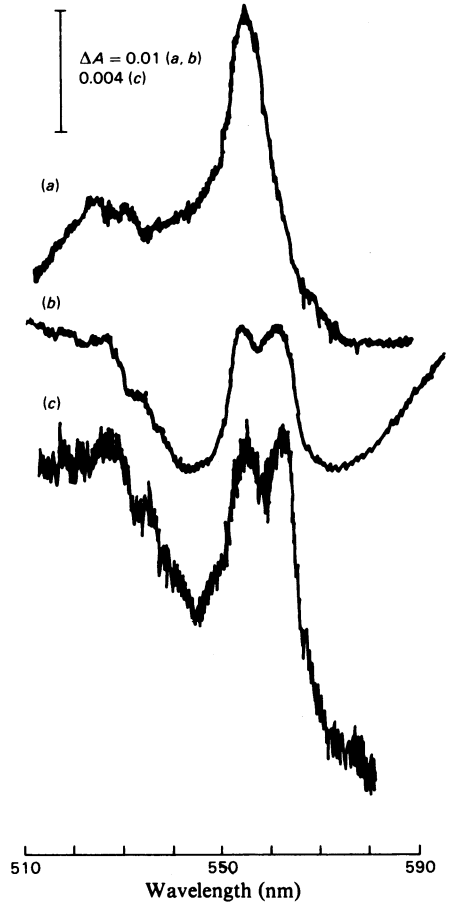

Fig. 4. *Difference spectra at 77K of membranes from aerobically grown E. coli poised at different electrode potentials*

Samples were prepared as described in the Materials and Methods section from suspensions flushed with argon (*a*, *b*) or with CO (*c*). The electrode potentials were as follows: (*a*) +2mV minus+134mV; (*b*) +180mV minus+350mV; (*c*)+130mV minus+320mV.

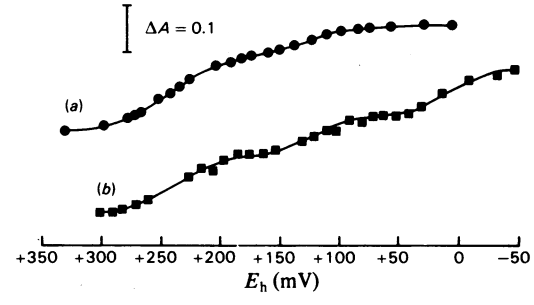

Fig. 5. *Redox titrations of the b cytochromes of membranes from anaerobically grown E. coli*

Electron-transport particles were derived from cells grown anaerobically with fumarate (*a*) or nitrate (*b*) and suspended in 50mM-Tes/50mM-KCl, pH7.0. The absorbance change was measured at 559nm. The solid lines are fitted for $n = 1$ for each component.

with E_{m7}+80mV has an α-band absorbance maximum at 556nm at 77K (Fig. 4*a*), and the high-potential absorbance change found during the titration in the absence of CO (Fig. 4*b*) is found also when CO is present (Fig. 4*c*). This confirms that the high-potential *b* cytochrome does not bind CO, whereas the other cytochrome *b* does. The high-potential *b* cytochrome apparently has a split α-band with maxima at 556 and 563nm. These observations confirm the finding that it is the lower-potential cytochrome *b* (E_{m7}+80mV) that binds CO and thus corresponds to cytochrome *o*.

Anaerobically grown cells

Cells grown anaerobically in the presence of fumarate with a non-fermentable carbon source (glycerol in the work described here) contain much higher concentrations of cytochromes a_1 and *d*, compared with aerobically grown cells (Fig. 1). The shape of the α-band of the *b*-type cytochromes is also very different in the two cell types. Redox

titrations of the *b* cytochromes of electron-transport particles derived from fumarate-grown cells (Fig. 5*a*) show the presence of two resolvable components, with E_{m7} values of +250mV and +140mV. Electron-transport particles derived from cells grown anaerobically with NO_3^- as terminal respiratory electron acceptor contain these two cytochromes and an additional cytochrome *b* with a mid-point potential of +10mV.

None of the *b* cytochromes found in anaerobically grown *E. coli* appears to bind CO; CO+dithionite minus dithionite-reduced difference spectra show no detectable bands in the 560nm region. Similarly, redox titrations of the *b* cytochromes of membranes from fumarate- and nitrate-grown *E. coli* are unaffected by the presence of CO.

The difference spectra of the individual *b* cytochromes of anaerobically grown cells were not completely resolved (Fig. 6), despite many attempts. This may be due to the temperature-dependence of mid-point potentials, since the samples were poised at a measured electrode potential at room temperature, then frozen to 77K. This does not usually present problems, since rates of electron transfer are not sufficiently rapid to allow redistribution of electrons at such low temperatures. It may be that the *b*-type cytochromes found in fumarate-grown cells re-equilibrate particularly rapidly, and therefore the two components are not completely separable by this method. However, it is clear from Fig. 6 spectra (*a*) and (*b*) that the peak at 558nm is relatively larger at higher potential, whereas the peak at 555nm is more significant at lower potential. This would suggest that the cytochrome *b* with a mid-point potential of +260mV has an α-absorbance maximum at 558nm and that the component at +140mV has an absorbance maximum at 555nm. In addition, dithionite-reduced-minus-oxidized difference spectra of electron-transport particles (Fig. 1*b*) show that the peak at 558nm is greater than that at 555nm. Redox titrations of these particles (Fig. 5*a*) show that the higher-potential cytochrome *b* contributes considerably more to the total spectral change than does the cytochrome *b* with an E_{m7} value of +140mV. This seems to confirm the suggested pairing of mid-point potentials and α-band absorbance maxima.

The cytochrome *b* titrating at E_{m7}+10mV in NO_3^--grown cells has an α-band maximum at 556nm (Fig. 6*c*).

Cytochromes a_1 and d

Typical redox titrations of cytochromes a_1 and *d* from fumarate-grown cells are shown in Fig. 7. Cytochrome *d* titrates as a single component with an E_{m7} value of +280mV. Cytochrome a_1 is resolved into two components contributing equally to the total spectral change with E_{m7} values of +260mV and +160mV. Redox titrations of cytochromes a_1 and *d* were also performed in the presence of CO. Cytochromes a_1 were still found to titrate at E_{m7}+260mV and +160mV, whereas all the cytochrome *d* bound CO and was not significantly oxidized by ferricyanide (E_h>450mV). The small amounts of cytochromes a_1 and *d* found in our electron-transport-particle preparations from aerobically grown cells were spectrally and thermodynamically identical with cytochromes a_1 and *d* found in preparations from cells grown anaerobically with fumarate or nitrate.

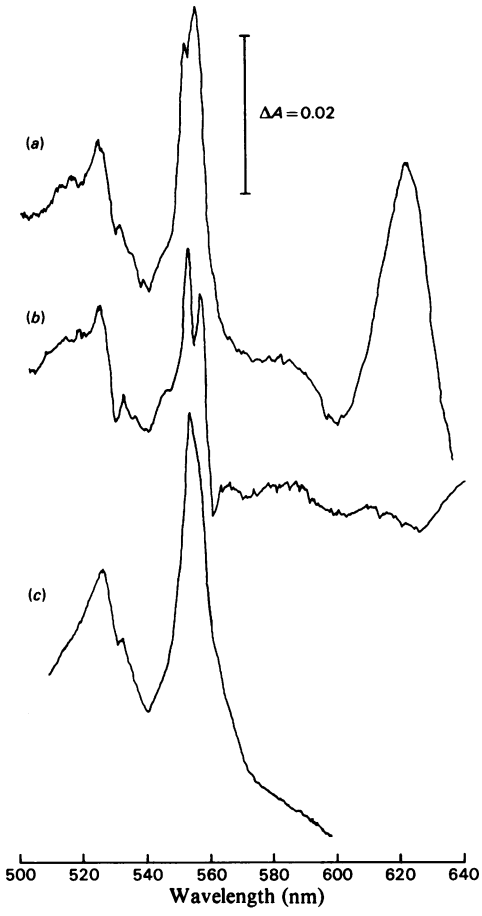

Fig. 6. *Difference spectra at 77K of membranes from anaerobically grown E. coli poised at different electrode potentials*

Samples were prepared as described in the Materials and Methods section from suspensions of particles from fumarate-grown (*a*, *b*) or nitrate-grown (*c*) cells. The cuvette was flushed with argon and the electrode potentials were as follows: (*a*)+240mV minus+350mV; (*b*)+100mV minus+195mV; (*c*) −260mV minus+30mV. The protein concentrations in the cuvettes were 7.8mg/ml (*a*, *b*) and 8.5mg/ml (*c*).

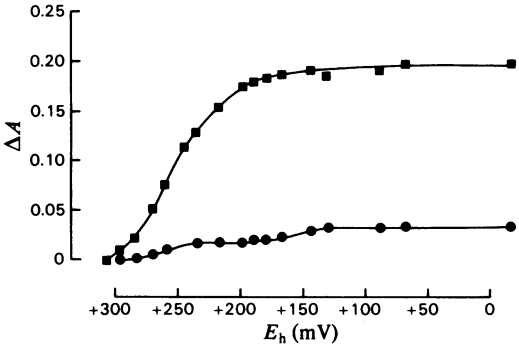

Fig. 7. *Redox titration of cytochromes a_1 and d*
Electron-transport particles were prepared from *E. coli* grown anaerobically with glycerol and fumarate, and suspended in 50mM-Tes/50mM-KCl, pH7.0. The reduction of cytochrome *d* (■) was measured by the absorbance increase at 630nm. Reduction of cytochrome a_1 (●) was estimated from the absorbance at 590nm. The solid lines are fitted for $n = 1$ for each component.

E. coli clearly has the capability of regulating the composition of its respiratory chain according to environmental conditions. This is particularly dramatic when the nature of the terminal respiratory oxidant is varied. Fumarate reductase and nitrate reductase are induced in the presence of fumarate and nitrate respectively under anaerobic conditions, but the electron-carrying pathways to O_2 (via cytochromes *o* and *d*) are also subject to regulation (Haddock & Jones, 1977). We have shown here that the respiratory-chain *b* cytochromes of aerobically and anaerobically grown *E. coli* are totally different. This latter regulation has not previously been studied; indeed the respiratory-chain components have not been clearly characterized. In this work we attempt to resolve the conflicting data on the cytochromes found in aerobically grown cells. The *b* cytochromes of anaerobically grown cells have not previously been analysed by potentiometry and we find these pigments to be distinct from the *b* cytochromes present in aerobically grown cells. The two sets of *b* cytochromes differ both in their mid-point redox potentials and in their spectrophotometric properties. By performing redox titrations in the presence of CO we have also shown which cytochromes bind CO, and are thus probably the oxidases.

The electron-transport pathway of aerobically grown cells terminates with cytochrome *o* (Castor & Chance, 1959), since these cells have no fumarate reductase or nitrate reductase and, when O_2 is readily available, no cytochrome *d*. The only cytochrome components of the respiratory chain under such conditions will be of type *b*. The low-potential cytochrome *b* ($E_{m7}-50$mV) is removed from the membranes by washing in buffer of low ionic strength and probably corresponds to the cytochrome b_1 crystallized by Deeb & Hager (1964). Electron-transport particles depleted of cytochrome *b* ($E_{m7}-50$mV) contain cytochromes *b* ($E_{m7}+80$mV and +260mV). It is tempting to suggest that one of these latter two cytochromes donates electrons to the other (cytochrome *o*), but we find that the probable cytochrome *o* has a mid-point potential some 180mV lower than the other cytochrome *b*. The physiological role of the high-potential cytochrome *b* is therefore unclear.

Cytochrome *o* is the sole oxidase of exponential-phase *E. coli* grown aerobically, but cytochromes a_1 and *d* are present in cells grown to stationary phase (Shipp, 1972), probably owing to a decrease in the steady-state O_2 concentration. We have shown here that cytochrome *d* readily binds CO, whereas cytochrome a_1 does not. Cytochrome *d* has been shown to have a higher affinity for O_2 than has cytochrome *o* (Rice & Hempfling, 1978), so its synthesis at low O_2 concentration provides an advantageous adaptive mechanism for this bacterium. Even when grown anaerobically with an alternative respiratory oxidant (fumarate or nitrate), *E. coli* synthesizes large amounts of cytochrome *d*, has high respiratory oxidase activity and exhibits O_2-dependent active transport of solutes (Boonstra *et al.*, 1975). The reason for the existence of such an extensive O_2-scavenging system is not obvious. *Azotobacter vinelandii* has an efficient mechanism for scavenging O_2 to protect its nitrogenase, which is inactivated by O_2 (Jones *et al.*, 1973); however, none of the enzymes of *E. coli* is known to have such an O_2 sensitivity.

The finding that cytochrome a_1 does not bind CO seems to rule out the possibility that this pigment is an oxidase in *E. coli*; it does, however, appear to have oxidase activity where found in other species (Ingledew, 1978). We have shown cytochrome a_1 in *E. coli* to contain two potentiometrically distinct haem centres. This feature is common to all those cytochromes a_1 (and aa_3) that have been investigated (Ingledew, 1978), and may be of functional importance. The function of cytochrome a_1 in the respiratory chain of *E. coli* remains a mystery, although it does appear to be co-induced with cytochromes *d* and b_{558} (see Haddock & Jones, 1977). The induction of cytochrome b_{555} in anaerobic systems has not been shown previously, presumably because of its spectral similarity to the cytochrome b_{556} component of aerobically grown cells.

Hendler *et al.* (1975) and Pudek & Bragg (1976) have also examined the cytochrome content of aerobically grown *E. coli*. The former resolved three *b* cytochromes with E_{m7} values of +220mV, +110mV and −50mV, in fairly close agreement with our findings (+260mV, +80mV and −50mV at pH7). Pudek & Bragg (1976) resolved two *b* cytochromes

with E_{m7} values of +165mV and +35mV, a profile that does not agree with our findings for aerobic electron-transport particles nor is compatible with a mixed population of aerobically and anaerobically induced *b* cytochromes. Pudek & Bragg (1976) also reported a single E_{m7} for cytochrome a_1 (+147mV), a further point of variance with our present findings.

E. coli grown anaerobically in the presence of nitrate produce large amounts of a *b* cytochrome, termed $b_{556}^{NO_3^-}$ (Ruiz-Herrera & De Moss, 1969), which is specifically oxidized by NO_3^-. We find that membranes from nitrate-grown cells contain a *b* cytochrome with a mid-point potential at +10mV and an α-band absorbance maximum at 556nm at 77K. This is not present in cells grown either aerobically or with fumarate anaerobically and is thus presumably cytochrome $b_{556}^{NO_3^-}$.

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