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The cytochrome *c* (552) of aerobically grown *Escherichia coli* str. McElroy and its function

When grown aerobically *Escherichia coli* has been found characteristically to contain cytochrome $d(a_2)$, a_1, *b* and *c* (ref. 1). In the course of growing *E. coli* str. McElroy for the preparation of cytochromes *d* and a_1 a cytochrome *c* having an α band at 552 mμ was detected in aerated cultures. When grown in the presence of helium (O_2 level 1/500000) this cytochrome *c* was the dominant cytochrome component in reflectance spectra, the cytochrome oxidases *d* and a_1 being barely detectable. When cultures were fully aerated the total amount of cytochrome *c* (552) increased but was less than the cytochrome *b*. When oxygen was totally excluded only a trace of cytochrome *b* could be found and cytochrome *c* (552) could not be detected even in broken cell preparations.

The inclusion of KNO_3 (0.5 %) in aerated cultures resulted in a marked decrease in the cytochrome *c* (552) content of the cells, as also that of the membrane oxidases. At a level of 2 % KNO_3, the oxidases were almost completely repressed and only a trace of the cytochrome *c* (552) could be detected in supernatants obtained by centrifuging at 120000 $\times$ *g* for 1 h. At the same time there was a marked decrease in the membrane fraction obtained from the broken cells. This suppressive effect of KNO_3 on the formation of membrane-bound oxidases has also been observed by us with *Aerobacter aerogenes* and *Haemophilus parainfluenzae*.

The cytochrome *c* was either released by rupture of the cells aerated in a Gifford Mini-mill at pH 7.4, or by extracting acetone-dried cells with 0.1 M sodium citrate at pH 6.6. Nucleic acids were precipitated with streptomycin and the extracts were chromatographed on columns of DEAE-cellulose equilibrated with 5 mM potassium phosphate buffer (pH 7.0) and eluted with a 40 mM concentration of the same buffer. Following chromatography on CM-cellulose equilibrated with 10 mM potassium phosphate (pH 6.2) the cytochrome *c* had a purity of 18 % (based on a molecular weight of 12000). By electrophoresis in starch the iso-electric point of the cytochrome *c* was estimated to be between 4.1 and 4.7. In the region pH 6.0–8.0 the cytochrome *c* separated into two zones, close together and of equal intensity. These two zones were detected in cytochrome *c* preparations from both highly aerated and micro-aerobic cells after a preliminary purification by a single passage of the extracts through a column of Sephadex G-100. Absorption maxima of the cytochrome *c* were 552 mμ (25.3), 523 mμ (15.0), 419 mμ (154.5) reduced; 523 mμ (9.2), 408 mμ (114.0) oxidized.

In whole cells some of the endogenously reduced cytochrome *c* (552) combined with CO and the percentage increased on breakage of the cells. Maintaining the cytochrome *c* at 50° for 4 h caused 78 % of the cytochrome to combine with CO at pH 7.0. The sensitivity of this cytochrome *c* (552) to alteration to a CO-combining form contrasts with the stability of the cytochrome *c* (552) obtained by FUJITA[2] from anaerobically grown *E. coli*. However, like the cytochrome *c* (552) obtained by these workers and that obtained by GRAY *et al.*[3], this cytochrome *c* (552) from aerobically grown *E. coli* str. McElroy, has a low oxidation–reduction potential (estimated as $E_0' = 150$ mV at 20°, pH 7.0, 0.05 M potassium phosphate buffer using the redox dye phenosafranine). The CO-combining form of cytochrome *c* (552) had the same oxidation–reduction potential as the native cytochrome.

In seeking a function for this cytochrome *c* (552) in aerated cells of *E. coli* str. McElroy it was thought that the cytochrome might be the specific electron donor to a peroxidase similar to that found in Pseudomonas sp. Considerable peroxidase activity was detected in extracts of *E. coli* str. McElroy when the assay of LENHOFF AND KAPLAN[4], which uses the dye 2,3',6-trichloroindophenol, was modified by using higher concentrations of H_2O_2. The specific activity of the extracts was increased three fold on dialysis against 0.8 mM phosphate buffer (pH 7.0) overnight. The peroxidase could not be measured with guaiacol as electron donor as the cells contained an enzymic activity which resulted in almost complete disappearance of the coloured product of peroxidation. Reduced horse-heart cytochrome *c* was not effective as an electron donor to the *E. coli* peroxidase. The peroxidase activity varied little over a range of growth conditions (Table I). Catalase activity was higher in the presence of nitrate, under which conditions it is expected that higher H_2O_2 concentrations would occur. The activity of *E. coli* catalase was stimulated rather than inhibited by 2,4-dichloroindophenol (0.4 mM) at which concentration beef-liver catalase is inhibited 100 %.

TABLE I

ACTIVITIES OF PEROXIDASE AND CATALASE IN *E. coli* (MCELROY)

Peroxidase was assayed by the method of LENHOFF AND KAPLAN[4]. H_2O_2, $3 \cdot 10^{-4}$ M, sodium azide, 10^{-2} M, extracts dialyzed for 24 h. Catalase activity was assayed iodometrically[5]. Medium: 5 g $(NH_4)_2HPO_4$, 1.15 g KH_2PO_4, 1 g $BaCl_2$, 0.1 $MgSO_4 \cdot 7\ H_2O$, 5 g peptone, 1 g Panmede per l distilled water, pH 7.4. Aeration with O_2 (95 %):CO_2 (5 %).

Growth conditions	*Peroxidase* ($A_{575\,m\mu}$ *change* $\times 10^2/30$ *sec per mg protein*)	*Catalase* ($K_{obs} \times 10^2$/*sec per mg protein*)
High aeration	50	1.2
Aeration by stirring	41	1.6
Under N_2 containing 0.2 % O_2	51	3.4
Under N_2 containing 0.001 % O_2	49	2.3
Under He containing 0.0002 % O_2	36	1.4
Still culture with KNO_3 2 %	40	4.2

Similar peroxidase activity was found in this organism under conditions where the formation of the cytochrome *c* was totally repressed. Peroxidase activity was also found in strains of *E. coli* and *Aerobacter aerogenes* in which no cytochrome *c* could be detected.

The following observations suggest that cytochrome *c* (552) is part of a sulphate-reducing system. Preparations in which cytochrome *c* was the major cytochrome were obtained from cells grown under He, broken and spun at 20000 $\times$ *g* for 20 min. The following salts (10 μmoles in 0.1 ml 0.1 M phosphate, pH 7.4) were added from the side arm of an evacuated Thunberg optical cell to the endogenously reduced supernatant, 3 ml: $NaNO_2$, KNO_3, Na_2SO_3, Na_2SO_4, NaCl. Both KNO_3 and $NaNO_2$ caused a change in the spectrum of the endogenously reduced cytochrome *c* (552) which was consistent with formation of a nitrite complex of the cytochrome. The addition of Na_2SO_4 or NaCl to the endogenously reduced extracts had no effect. Immediately following addition of Na_2SO_3, the α band at 552 mμ was observed with a hand

spectroscope to disappear then quickly reappear, being always at the level of intensity of the band in the control endogenously reduced solution by the time the spectrum was recorded. This cycle of endogenous reduction–oxidation–reduction was found to be reproducible with further additions of sulphite. The ability of the cytochrome *c* to be cyclically oxidized by Na_2SO_3 was lost when particulate material was removed by centrifuging at 120000 × *g* for 1 h. To further eliminate the possibility of non-enzymatic oxidation the effect of making the extracts 10 mM with respect to NaCN was examined. No oxidation was now obtained upon the subsequent addition of Na_2SO_3, $NaNO_3$ or $NaNO_2$. Addition of NH_2OH (10 μmoles) resulted in complete oxidation of the cytochrome regardless of the presence of cyanide.

There is thus some evidence that in aerobic cells this cytochrome *c* (552) could be involved in a subsidiary energy-yielding pathway in which sulphite is the terminal electron acceptor. The system is regarded as vestigial as in this organism the ability to form the cytochrome *c* under aerobic conditions is rapidly lost if the organism is maintained in medium not containing a high sulphur content.

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Inhibitors of NADH–ubiquinone reductase from mitochondria

It has been reported[1,2] that very brief treatment of submitochondrial particles with low concentrations of *Naja naja* venom abolishes the physiological, fully rotenone-sensitive NADH–ubiquinone (NADH–Q) reaction. If the treatment was brief, washing with serum albumin followed by the addition of phospholipids restored the reaction completely. These results were interpreted to suggest that the inactivation by the venom was due in part to the formation of an inhibitor, in part to the cleavage of phospholipids which may be required for the reduction of exogenous ubiquinone in intact systems.

Recently, it was reported[3,4] that brief incubation of heavy electron transport particle (ETP_H) with *N. naja* venom not only abolishes its Q-6 reductase activity

Abbreviations: ETP, electron transport particle; ETP_H, heavy electron transport particle.