The Respiratory Chain of a Newly Isolated *Methylomonas* P11

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1. Whole cells of *Methylomonas* P11 contained ubiquinone, identified as ubiquinone-8. No naphthaquinone was detected. Ubiquinone was located predominantly in the particulate fraction, which also contained most of the NADH oxidase activity. 2. Aerobic incubation of cells with formaldehyde or methanol resulted in about 20% reduction of ubiquinone, irrespective of the presence or absence of dinitrophenol. On inhibition of the respiration by cyanide, ubiquinone became partly reduced by endogenous substrates (15–25%), and a further reduction occurred only in the presence of formaldehyde (up to 60%). When endogenous substrates were completely exhausted, then 44 and 23% of ubiquinone was reduced by formaldehyde or methanol respectively. 3. The difference spectra at room and liquid-N₂ temperatures revealed the presence of cytochrome *b* and two cytochromes *c* (*c*-552.5 and *c*-549) all tightly bound to the membrane. Cytochrome *c*-552.5 was also found in the soluble fraction. 4. Redox changes of cytochromes *b* and *c*, with methanol or formaldehyde as substrates, respond to the aerobic and anaerobic states of the cell and to KCN inhibition in a manner characteristic of the electron carriers of the respiratory chain. 5. The merging point for electron transport from NADH dehydrogenase and formaldehyde dehydrogenase is suggested to be at the level of ubiquinone.

In the last few years the respiratory chain of methanol-utilizing bacteria has been subjected to many investigations (Anthony, 1970; Davey & Mitton, 1973; Tonge *et al.*, 1974; Widdowson & Anthony, 1975).

The primary dehydrogenases oxidizing methanol, methylamine, formaldehyde and formate have been isolated from both obligate and non-obligate methylotrophs and partially characterized (Johnson & Quayle, 1964; Patel *et al.*, 1972; Wadzinski & Ribbons, 1975). However, participation of the particular respiratory-chain components in oxidation of methanol and other C₁ substrates has not been closely investigated.

Widdowson & Anthony (1975), studying the oxidation processes in whole cells of the facultative methylotroph *Pseudomonas* AM1 and its cytochrome *c*-deficient mutant, have concluded that cytochrome *b* is the first component involved in the electron-transport system for oxidation of all substrates except methanol. According to these authors methanol dehydrogenase of *Pseudomonas* AM1 merges into the oxidation system via cytochrome *c*.

In the present paper the quinone and some components of the cytochrome system of a newly isolated methanol obligate (Raczyńska-Bojanowska *et al.*, 1977) are identified. This organism is able to utilize methanol, but does not grow on methylamine or methane like the obligate methylotroph *Methylomonas* W1 isolated by Dahl *et al.* (1972). The dehydro-

genase is a pteridine-type enzyme, phenazine methosulphate-dependent and catalyses oxidation of both methanol and formaldehyde (Michalik & Raczyńska-Bojanowska, 1976). Spectral evidence for the occurrence of two species of cytochrome *c* and one species of cytochrome *b* is presented. The redox reaction of cytochrome *c* and cytochrome *b* in response to the addition of methanol or formaldehyde as substrates indicate that both cytochromes are involved in electron transport.

The function and position of ubiquinone in the respiratory chain of *Methylomonas* P11 is discussed.

Materials and Methods

Organism

An obligate methylotroph, *Methylomonas* ZB36 P11 Mut-11, has been isolated by the enrichment technique from activated sludge (Raczyńska-Bojanowska *et al.*, 1977).

Growth conditions

Methylomonas P11 was grown in 2-litre Erlenmeyer flasks containing 1 litre of mineral medium described by Tonge *et al.* (1974) with 1% (v/v) methanol as sole carbon source. The medium was inoculated with 10 ml of cell suspension (*A*₆₅₀ = 2) and incubated on a rotary shaker at 30°C for 24 h. The cells were harvested

in the late exponential phase of growth by centrifugation at 10000g, for 10 min, washed twice with 20 mM-sodium/potassium phosphate buffer, pH 7.0, and suspended in the same buffer to yield a heavy suspension (40–50 mg of protein/ml).

Preparation of particulate fraction

The suspension of cells (100 mg wet wt./ml of 20 mM-sodium/potassium phosphate buffer, pH 7.0) was sonicated for 7×2 min in a MSE ultrasonic disintegrator at 20 kHz in an ice bath and centrifuged for 10 min at 10000g to yield the cell-free supernatant. The particulate fraction was then separated by centrifugation for 3 h at 140000g. The clear supernatant obtained (supernatant I) was carefully separated from the particulate fraction (particulate fraction I) and dialysed against 20 mM-sodium/potassium phosphate buffer, pH 7.0, overnight. The particulate fraction I was suspended in 10 mM-sodium/potassium phosphate buffer, pH 7.0, to yield about 50 mg of protein/ml, and was either frozen or stored at 2°C. Particulate fraction II was obtained as follows. Particulate fraction I suspended in 50 mM-sodium/potassium phosphate buffer, pH 7.0, was sonicated for 2×2 min in MSE ultrasonic disintegrator at 20 kHz in an ice bath. The resulting homogenate was centrifuged, as described for the separation of particulate fraction I, giving supernatant II and particulate fraction II.

Isolation and identification of quinones

The cells separated from the culture medium either directly or after freeze-drying were extracted three times with methanol/chloroform (2:1, v/v) as described by Kates (1972). The extract was evaporated to dryness in a rotary evaporator, and lipid residue was immediately dissolved in chloroform/methanol (1:1, v/v). The extract was chromatographed on silica-gel G plates in light petroleum (b.p. 40–60°C)/chloroform (4:1, v/v) as the solvent system. Quinones were localized under u.v. illumination, quickly scraped off and extracted with 3×2–3 ml of ethyl ether. The extracts were collected and concentrated under vacuum. Purification on silica-gel plates was repeated twice. The purified quinone was identified by reversed-phase t.l.c. on silica-gel plates impregnated with 5% (v/v) liquid paraffin in light petroleum (b.p. 40–60°C). The plates were developed in the two following solvent systems: acetone/water (19:1, v/v) or ethanol/water (19:1, v/v). The plates were stained for quinones with reduced Methylene Blue in ethanol. A sample of chromatographically purified quinone was dissolved in ethanol, and the difference spectrum (reduced with KBH_4 minus oxidized) was recorded in the range 240–320 nm by using a Cary model 118 spectrophotometer.

The content of ubiquinone in cells and subcellular fractions and the degree of its reduction in different metabolic states were determined by the extraction procedure originally introduced by Pumphrey & Redfearn (1960) and slightly modified by Drabikowska & Kruszewska (1972). A mixture of 7.5 ml of methanol and 3 ml of light petroleum (b.p. 40–60°C) was added to 2 ml of the cell suspension and vigorously shaken for 1 min. The phases were separated by centrifugation for 3 min at 4000g. The upper layer was removed and the extraction was repeated twice. The pooled extracts were washed with 2 ml of 95% methanol and centrifuged as above. The upper layer was transferred to a beaker and evaporated to dryness at 50°C under N_2 . The residue was dissolved in 1.5 ml of absolute ethanol. The ubiquinone content was estimated from the decrease in $A_{275} - A_{294}$ after reduction of chromophore with KBH_4 . The amount of ubiquinone was calculated with the use of the millimolar absorption coefficient of $12.25 \times 10^{-3} \text{ cm}^{-1}$. All the experiments were carried out with cells in which endogenous substrates were greatly diminished by a thorough aeration of the diluted cell suspension (0.6–1.2 mg of protein/ml). The amount of endogenous substrates was evaluated by measurements of O_2 consumption and/or by the degree of ubiquinone reduction after inhibition of the respiration by cyanide.

Spectrophotometric analysis

Difference spectra of cytochromes at room temperature were recorded in the Cary 118 spectrophotometer. The low-temperature spectra were recorded in the split-beam Hitachi spectrophotometer. The absorption coefficients (reduced minus oxidized) were assumed to be similar to those of mammalian cytochromes: for cytochrome *c* $\epsilon_{550-540 \text{ nm}} = 18700 \text{ M}^{-1} \cdot \text{cm}^{-1}$, and for cytochrome *b*, $\epsilon_{562-575 \text{ nm}} = 23400 \text{ M}^{-1} \cdot \text{cm}^{-1}$.

The redox states of the *c*- and *b*-type cytochromes in response to aerobic and anaerobic conditions were measured in the Hitachi dual-wavelength spectrophotometer.

Oxygen consumption

The oxygen consumption was assayed polarographically by using a Clark oxygen electrode. The reaction mixture contained: 25 mM-Hepes* buffer, pH 7.0, the particulate or the whole-cell fraction, and the substrate at 5 mM concentration.

Protein determination

Protein was determined by the biuret method described by Drabikowska (1975).

* Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

Results

Respiration

Cells of *Methylomonas* P11 contain considerable amounts of endogenous substrates which are actively oxidized and completely saturate the respiratory chain with reducing equivalents. Thus on the addition of any substrate investigated no stimulation of respiration could be observed. Therefore the measurements of respiration with appropriate substrates were preceded by thorough aeration of cell suspension to exhaust endogenous substrates (see the Materials and Methods section). Then the respiration rate of intact cells of *Methylomonas* P11 with formaldehyde or methanol as substrates was 230 and 250 nmol of O_2 /min per mg of protein respectively.

Respiration with either methanol or formaldehyde as substrate was inhibited completely by 1 mM-cyanide.

The particulate fraction catalysed only NADH oxidation. The primary methanol dehydrogenase and formaldehyde dehydrogenase were found only in the supernatant. Thus one may assume that they were either located in the cytoplasm or were released from the cell membrane by sonication.

Components of the respiratory chain

Quinones. *Methylomonas* P11 had no menaquinone and only one kind of ubiquinone, namely ubiquinone with eight isoprenoid units. Identification was based on the u.v. spectrum, which was similar to that of ubiquinone-8 or -10, with a maximum at about 274–275 nm. In addition to this evidence the migration rate of the isolated quinone in reversed-phase t.l.c. was compared with that of standard ubiquinones-6, -8 and -10 (Fig. 1). The quinone isolated from *Methylomonas* P11 migrates together with ubiquinone-8 in ethanol/water and acetone/water as solvent systems. When mixed with ubiquinone-8 it could not be chromatographically separated.

The content of ubiquinone in cells and subcellular fractions is presented in Table 1. The content of ubiquinone per mg of protein in the particulate fraction, separated from the sonic extract by centrifugation at 140 000g, was almost twice as high as in the whole cells. Ubiquinone present in the particulate fraction or in the cells vigorously aerated before extraction was exclusively in the oxidized form.

The ubiquinone/cytochrome *b* ratio was the same in the whole cells and the particulate fractions (Table 1), indicating that ubiquinone is located

Fig. 1. T.l.c. of quinones isolated from lipid extract of *Methylomonas* P11 cells

Silica-gel G plates were impregnated with a solution of 5% paraffin in light petroleum (b.p. 40–60°C): (a) developed in acetone/water (19:1, v/v); (b) developed in ethanol/water (19:1, v/v). Plates were stained by spraying with ethanolic solution of reduced Methylene Blue. Ubiquinones-6, -8, -10 and vitamin K were used as standards. I, Test sample.

Table 1. Concentrations of ubiquinone and cytochromes *b* and *c* in the cells and subcellular fractions of *Methylomonas* P11

The contents of cytochromes *b* and *c* were calculated from measurements by the dual-wavelength spectrophotometer, by using the absorption coefficients given in the Materials and Methods section. In the calculation of cytochrome *b* concentration the interference of cytochrome *c* at $A_{562}-A_{575}$ was taken into account. Ubiquinone content was determined by the extraction procedure of Drabikowska & Kruszewska (1972).

Preparation	Content (nmol/mg of protein)			Ubiquinone	Cytochrome <i>c</i>
	Ubiquinone	Cytochrome <i>c</i>	Cytochrome <i>b</i>	Cytochrome <i>b</i>	Cytochrome <i>c</i>
Cells	1.4	2.0	0.27	5.2	7.3
Particulate fraction I	2.1	1.3	0.38	5.7	3.5
Particulate fraction II	2.5	1.4	0.43	5.7	3.2
Supernatant I	0.2	2.5	—	—	—
Supernatant II	0.0	0.3	—	—	—

Fig. 2. Room-temperature difference spectrum of whole cells. Cell samples (1.57 mg of protein/ml) suspended in 10 mM-sodium/potassium phosphate buffer, pH 7.0, reduced with 5 mM-methanol or 5 mM-formaldehyde, and oxidized with a drop of H_2O_2 , were placed in the test and reference cuvette respectively. Optical path-length 1 cm.

exclusively in the cell membrane together with tightly bound cytochrome *b*.

The small amount of ubiquinone found in the soluble fraction may be derived from small membrane fragments that did not sediment on centrifugation.

Cytochromes. The difference spectra of cells (reduced by methanol versus oxidized by H_2O_2) are given in Fig. 2. The peak in the α -region at 552.5 nm could be attributed to cytochrome *c*. At the shoulder of cytochrome *c* a small peak at 562 nm, not well separated, indicates, however, the presence of cytochrome *b*. An additional, hardly perceptible, peak at 549 nm may occasionally be observed (not shown in Fig. 2). This might suggest the occurrence of another cytochrome *c*. In the β -region,

Fig. 3. Low- and room-temperature difference spectra of the soluble fraction.

Soluble fraction I (2.1 mg of protein/ml) reduced with dithionite and oxidized with H_2O_2 was placed in the test and reference cuvette respectively. (a) Room-temperature spectrum; (b) α - and β -region of spectra of cytochromes recorded at liquid- N_2 temperature.

there were bands at 523 and 530 nm attributable to cytochromes *c* and *b* respectively. Only one absorption band at 422 nm appeared in the Soret region. This band probably comprised the non-resolved bands of at least two cytochromes *c* and cytochrome *b*. A similar spectrum was obtained when formaldehyde was used as substrate.

The room-temperature difference spectrum of the membrane fraction (not shown) indicated clearly the existence of two closely neighbouring peaks (552.5 and 549 nm) and a well-separated peak at 562 nm, confirming the occurrence of two species of cytochrome *c* and one of cytochrome *b*.

Fig. 4. Low-temperature difference spectra of the particulate fractions

The particulate fractions were suspended in 10mM-sodium/potassium phosphate buffer, pH7.0. In all cases the reference sample was kept aerobic, whereas the test sample was reduced by dithionite. (a) Particulate fraction I (2mg of protein/ml), optical path-length 1 mm. (b) Particulate fraction II (1.3mg of protein/ml), optical path-length 2 mm.

Fig. 5. Low-temperature difference spectra of whole cells

Cell samples suspended in 10mM-sodium/potassium phosphate buffer, pH7.0 (4.0mg of protein/ml), reduced with 5 mM-methanol and oxidized with a drop of H_2O_2 were placed in the test and reference cuvette respectively.

In the soluble fraction cytochrome *c*-552.5 was the only cytochrome compound found (Fig. 3). The presence of cytochrome *c*, showing the same difference spectrum, in the particulate and the soluble fractions suggested that cytochrome *c*-552.5 was only partly released from the membrane to the supernatant during cell disruption. Thus it might be expected that cytochrome *c*-552.5 remaining in the cell membrane could be removed by washing with phosphate buffer or by resonication of the particulate fraction. However, I have not succeeded in removing cytochrome *c* from the particulate fraction either by washing particles with 20 or 50 mM-phosphate buffer or by resonication. This is evident from the ratio of cytochrome *c*/cytochrome *b* or cytochrome *c*/ubiquinone having the same value in both particulate fraction (Table 1). I cannot explain this phenomenon, but assume that cytochrome *c*-552.5 present in the particulate fraction is tightly bound to the cell membrane, together with other respiratory-chain components. Moreover, results presented in Fig. 4 indicate that neither of the two species of cytochrome *c* can be removed from the particulate fraction I, as the ratios of particular cytochromes in the subsequent preparations remain constant (calculated from their peak heights).

For more accurate determination of cytochrome components in whole cells and subcellular fractions the difference spectra were taken at liquid- N_2 temperature (Fig. 5). A higher resolution of the complex spectra of *c*-type cytochromes in all fractions studied was achieved, with a small shift to the lower wavelength. In the whole cells and the particulate fraction two cytochromes *c* with absorption bands in the α -region at 549 nm and 547 nm were well distinguished. The absorption band of cytochrome *b* at 560 nm was well separated from those of cytochromes *c*. I also succeeded in the resolution of the spectrum in the Soret region into two peaks at 422 nm and 416 nm (Fig. 6).

In the soluble fraction only cytochrome *c*, with a peak at 549 nm corresponding to that found in the particulate fraction, was present (Fig. 3).

Participation of ubiquinone and cytochromes in the oxidation of methanol and formaldehyde

Ubiquinones. Either methanol or formaldehyde when added to the highly aerated cell suspension reduced about 20% of the ubiquinone (Table 2). This reduction was not caused by energy-dependent reactions, since uncoupling by 2,4-dinitrophenol did not change the steady-state reduction of ubiquinone. On inhibition of respiration by cyanide, in most cases about 20–30% of ubiquinone became reduced as a result of endogenous substrate oxidation. Addition of formaldehyde as substrate increases the reduction of ubiquinone by up to

Fig. 6. Low-temperature difference spectra of particulate fraction I

Samples contained 5.1 and 7.0 mg of protein/ml of particulate fraction I suspended in 10 mM-sodium/potassium phosphate buffer, in (a) and (b) respectively. The test cuvette contained particulate fraction reduced by dithionite, the reference was oxidized by H_2O_2 . (a) Optical path-length 2 mm; (b) optical path-length 3 mm.

Table 2. Redox changes of ubiquinone in cells and the particulate fraction I

Assays were carried out as described in the Materials and Methods section. The particulate fraction I (2.6 mg of protein/ml) and cells (0.8–1.7 mg of protein/ml) were suspended in 10 mM-Hepes buffer, pH 7.0. The reaction was started by the addition of substrate to the vigorously aerated reaction mixture: 1.0 mM-NADH; 5 mM-formaldehyde; 5 mM-glyceraldehyde or 5 mM-methanol were used as substrates. Incubation was carried out for 30 s at room temperature. The total volume was 2 ml. Where indicated 2 mM-KCN was added 30 s before the addition of substrate. Ubiquinone content in the cells and the particulate fraction was 1.3–1.4 and 3.1 nmol/mg of protein respectively.

Preparation	Expt. no.	Substrate added	Ubiquinone reduced (%)			
			-2,4-Dinitrophenol		+2,4-Dinitrophenol	
			-KCN	+KCN	-KCN	+KCN
Cells	1	None	0	29	0	19
		Formaldehyde	—	56	—	57
		Methanol	—	29	—	19
Cells	2	None	0	0	—	—
		Formaldehyde	—	44	—	—
		Glyceraldehyde	—	44	—	—
		Methanol	—	23	—	—
Cells	3	None	0	23	0	—
		Formaldehyde	18	39	18	—
		Methanol	15	23	15	—
Particulate fraction I	1	None	0	0	—	—
		NADH	26	51	—	—

60%. In such conditions methanol did not affect the degree of ubiquinone reduction over that resulting from the endogenous substrate oxidation. When the endogenous substrates had been completely exhausted by a long aeration (Table 2, Expt. 2), the reduction of ubiquinone caused by formaldehyde, as well as by glyceraldehyde, was as high as 44%, whereas that by methanol was only 23%.

To see whether the lack of complete ubiquinone

reduction in the cells is due to limitation of substrate availability, the particulate fraction was used. NADH was the only substrate oxidized by these preparations, since the primary dehydrogenases oxidizing methanol or formaldehyde probably remained in the soluble fraction. The reduction of ubiquinone by NADH in the particulate fraction (see Table 2) was very similar to that observed in the whole cell. Thus one may assume that only about 60% of the total amount of

Fig. 7. Redox changes of cytochromes *b* and *c* in the whole cells

Cells were suspended in 10 mM-Hepes, pH 7.0. Where indicated, 5 mM-methanol and 1 mM-KCN were added to the aerobic cell suspension. (a) Redox changes of cytochrome *b* measured at $A_{562}-A_{575}$ (0.7 mg of protein/ml); (b) redox changes of cytochrome *c* at $A_{550}-A_{540}$ (0.43 mg of protein/ml). The complete reduction was achieved by the addition of a few crystals of dithionite. Optical path-length 1 cm.

ubiquinone present in the cell is accessible to enzymic reduction, and that only that part participates in electron transport.

Cytochromes. The participation of cytochromes *b* and *c* in the respiration of methanol or formaldehyde was investigated by direct spectrophotometric recording of their redox reactions under different metabolic states of intact cells. The redox states of cytochromes (Fig. 7) demonstrate an immediate increase in absorbance on addition of any substrate tested, corresponding to the oxidation-reduction state of both cytochromes *c* and *b* in the aerobic conditions and their further reduction either on transition to anaerobiosis or on inhibition of respiration by KCN.

To rule out the possibility that the reduction of cytochromes in the kinetic experiments described above is due to reversal of electron transport driven by ATP or energy-rich intermediates, which might be present in the cell, the same experiments were carried out in the presence of 2,4-dinitrophenol, which effectively uncouples the oxidative phosphorylation and thus prevents the energy-dependent processes. Similar results obtained in the presence or absence of 2,4-dinitrophenol indicate that the reduction of cytochromes results directly from the oxidation of substrate. The reduction of cytochromes in the whole cells in anaerobic conditions or on inhibition by KCN may result from the presence of endogenous substrates. As shown in Fig. 8 (trace *a*)

Fig. 8. Room-temperature difference spectrum of the whole cells after thorough aeration (10h)

Cell samples (0.9 mg of protein/ml) were suspended in 10 mM-Hepes buffer, pH 7.0. (a) Test sample was reduced by endogenous substrates after addition of 1 mM-KCN; (b) 5 min after the addition of KCN (1 mM), 5 mM-methanol was added.

even after thorough aeration (10h) the reduction of cytochromes occurred on addition of KCN. However, a further reduction of cytochromes *b* and *c* (trace *b*) on the addition of methanol or formaldehyde is indicative of their participation in oxidation of these substrates.

Discussion

The present results demonstrate that ubiquinone with eight isoprenoid units is the major quinone present in *Methylomonas* P11. The absence of menaquinone from aerobically grown bacteria agrees with the observation of Bishop *et al.* (1962), who found that Gram-negative bacteria contain ubiquinone but no menaquinone.

The localization of ubiquinone together with cytochromes and NADH dehydrogenase in the membrane of the *Methylomonas* P11 suggests the involvement of ubiquinone in electron transport. The redox reactions of ubiquinone and its response to KCN inhibition indeed indicate it to be an obligate member of the respiratory chain oxidizing formaldehyde of NADH in *Methylomonas* P11. A similar degree of ubiquinone reduction by formaldehyde or glyceraldehyde in the whole cells and by NADH in the particulate fraction indicates that the pool of ubiquinone is equally accessible to the reducing equivalents provided by these substrates, and stresses the role attributed to ubiquinone as the reducing-equivalent-collecting pool in the respiratory chain (Szarkowska & Klingenberg, 1963; Kröger & Klingenberg, 1966). On the contrary, the reason for

only slight reduction of ubiquinone with methanol is unclear at present, the more so as the 20-fold purified methanol dehydrogenase oxidizes methanol and formaldehyde as well (Michalik & Raczyńska-Bojanowska, 1976).

The high ubiquinone/cytochrome *b* ratio is comparable with that found in the mammalian and bacterial respiratory system, for which the function of ubiquinone as an obligatory electron-transport component has been established (Kröger & Klingenberg, 1966; Szarkowska, 1966; Benziman & Goldhamer, 1968).

The cytochrome system of *Methylomonas* P11 described in the present paper was composed of cytochrome *b* and two cytochromes of *c*-type (552.5 and 549 nm); one of them (cytochrome *c*-549) has not been demonstrated so far in other *Methylomonas* species. Cytochrome *c*-549 is firmly bound to the membrane-like cytochrome *c*₁ in mammalian mitochondria. Cytochrome *c*-552.5 seems to be, at least partly, tightly bound to the membrane.

The difference spectrum of cytochrome *c* present in the soluble fraction exactly resembles that of cytochrome *c*-552.5 in the particulate fraction. Perhaps some of this cytochrome *c* is less tightly bound and is removed from the membrane during sonication. One cannot, however, exclude the possibility that this cytochrome *c* occurs in a soluble form in the cells as well. The similarity of cytochrome *c* present in the soluble and the particulate fraction of *Methylomonas* P11, as suggested by difference spectra, cannot fully prove their identity. Therefore, as long as the difference between cytochrome *c* present in the supernatant and in the particulate fraction is not well documented, we may consider them as identical. The occurrence of a soluble cytochrome *c* in *Pseudomonas* AM1 and its mutants has been reported by Anthony (1975). Cytochrome *c* does not normally occur in the cytoplasm. However, exceptional circumstances have been described for the Poky strain of *Neurospora crassa* by Scott & Mitchell (1969), who have found a 16-fold accumulation of cytochrome *c*, half of which was in the soluble form. Thus it may be supposed not only that this phenomenon is characteristic of the respiration-deficient mutants of *Neurospora* but that it also occurs in bacteria cultured on carbon sources such as methanol or other C₁ compounds. It is noteworthy that although cytochrome *c* in *Methylomonas* P11 is present at an unusually high concentration and is responsible for the pink colour of these organisms, the whole amount of cytochrome *c* participates in the enzymic oxidation of methanol or formaldehyde.

The aerobic steady-state reduction of cytochrome *b* and *c* in the presence of a respiratory substrate such as methanol or formaldehyde, as well as their further reduction in response to inhibition of respiration by

cyanide, are characteristic of respiratory-chain components.

According to Widdowson & Anthony (1975), cytochrome *c* is the first acceptor for methanol dehydrogenase in the respiratory chain of *Pseudomonas* AM1, whereas other dehydrogenases merge into respiratory chain at the level of cytochrome *b*.

Results in the present paper indicate that in *Methylomonas* P11 formaldehyde and NADH are oxidized by dehydrogenases which join the respiratory chain at the level of ubiquinone. Both cytochrome *b* and *c* are components of the respiratory chain of *Methylomonas* P11 which participate in oxidation of methanol, formaldehyde and NADH.

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