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CYTOCHROME *c* INVOLVED IN THE REDUCTIVE DECOMPOSITION OF ORGANIC MERCURIALS

PURIFICATION OF CYTOCHROME *c*-I FROM MERCURY-RESISTANT *PSEUDOMONAS* AND REACTIVITY OF CYTOCHROMES *c* FROM VARIOUS KINDS OF BACTERIA

KENSUKE FURUKAWA and KENZO TONOMURA

Fermentation Research Institute, Ministry of International Trade and Industry, Inage, Chiba, 281 (Japan)

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SUMMARY

Cytochrome *c*-I which was involved in the decomposition of organic mercurials as an electron carrier was purified from the cell-free extract of the mercury-resistant strain, *Pseudomonas* K62, by means of $(NH_4)_2SO_4$ precipitation and column chromatography on Sephadex G-150, DEAE-Sephadex and Sephadex G-75. The cytochrome was crystallized in a needle-like form. It showed absorption maxima at 550, 521, and 416.5 nm in the reduced form, and the pyridine ferrohemochrome had absorption maxima at 549, 520, and 413 nm, suggesting it to be a *c*-type cytochrome.

Cytochromes *c* prepared from type cultures of bacteria belonging to the genera *Aeromonas*, *Micrococcus*, *Bacillus*, *Corynebacterium*, *Staphylococcus*, *Aerobacter*, and *Pseudomonas* were all inactive with respect to the decomposition of phenylmercuric acetate. However, cytochrome *c* prepared from *Pseudomonas* CF, which was isolated from the activated sludge acclimatized with $HgCl_2$ and phenylmercuric acetate, as well as the cytochrome *c*-I of *Pseudomonas* K62, were active in this respect.

INTRODUCTION

In previous papers[1–4], the authors have revealed the decomposition mechanism of mercurials by an enzyme system obtained from mercury-resistant *Pseudomonas* K62. Phenylmercuric acetate, methylmercuric chloride, ethylmercuric phosphate and $HgCl_2$ were all reduced to metallic mercury by the system consisting of D-glucose:NAD(P) oxidoreductase (EC 1.1.1.47, glucose dehydrogenase) or L-arabinose:NADP oxidoreductase (EC 1.1.1.46, arabinose dehydrogenase) as a NAD(P)H-generating system, a metallic mercury-releasing enzyme which possesses FAD as a prosthetic group, and one of the *c*-type cytochromes (cytochrome *c*-I). Metallic mercury-releasing enzyme was specifically induced by addition of mercurials such as phenylmercuric acetate, $HgCl_2$, *p*-chloromercuribenzoate, while glucose dehydrogenase, arabinose dehydrogenase and cytochrome *c*-I were constitutive.

Two kinds of cytochrome *c* (*c*-I and *c*-II) were found in the cell extract of this organism[2]. Cytochrome *c*-I with a mol. wt of 26 000, was active in the decomposition, while cytochrome *c*-II, with a mol. wt of 14 000, was inactive However, cytochrome *c*-I has not yet been purified thoroughly. The present paper describes the crystallization of cytochrome *c*-I and examination of some of its properties, and reactivity of cytochromes *c* prepared from various kinds of bacteria in the decomposition of phenylmercuric acetate. It was found that one of the *c*-type cytochromes from *Pseudomonas* CF which was newly isolated from activated sludge was able to couple in the reaction as well as the cytochrome *c*-I. The role of cytochrome *c*-I is discussed in comparison with its role in the respiratory chain

MATERIALS AND METHODS

Microorganisms and cultivation

A mercury-resistant strain, K62, of *Pseudomonas* was isolated from the soil of a phenylmercuric acetate-producing factory previously[5]. The composition of the culture medium (Medium A) for this organism was as follows: glucose, 2.5 g; KH_2PO_4, 0.2 g; K_2HPO_4, 1.6 g; $(NH_4)_2SO_4$, 1 g; $MgSO_4 \cdot H_2O$, 0.2 g; $CaCl_2 \cdot 2\,H_2O$, 0.02 g; NaCl, 0 1 g; $FeSO_4 \cdot 7\,H_2O$, 0.01 g; Na_2MoO_4, 0.5 mg; $MnSO_4$, 0.5 mg; calcium pantothenate, 0.4 mg; inositol, 0.2 mg; nicotinic acid, 0.4 mg; riboflavin, 0.4 mg; biotin, 2 μg; vitamin B_{12}, 0.5 μg; phenylmercuric acetate 30 mg; and distilled water, 1 l (pH 7.5). Cultivation was carried out with shaking at 30 °C. *Aeromonas hydrophila*, *Micrococcus luteus*, *Bacillus subtilis*, *Corynebacterium equi*, *Staphylococcus aureus*, *Aerobacter aerogenes*, *Pseudomonas fluorescens*, *P. aeruginosa*, *P. ovalis*, *P. riboflavina* and *P. mephitica var. lipolitica* were aerobically grown on a medium (Medium B) containing meat extract, 7 g; peptone, 10 g; NaCl, 3 g; $FeSO_4 \cdot 7\,H_2O$, 0.02 g; and distilled water, 1 l (pH 7.0) at 30 °C. The CF strain of *Pseudomonas* was newly isolated from the activated sludge which was acclimatized with $HgCl_2$ and phenylmercuric acetate. The organism was aerobically grown on Medium B supplemented with 20 mg phenylmercuric acetate.

Preparation of cell extract

After cells were harvested and washed with 0.05 M phosphate buffer (pH 6 7), they were disrupted by shaking with glass beads (0.10–0.11 mm diameter) using a Braun Cell Homogenizer. The homogenate was centrifuged at 15 000 $\times g$ for 30 min. The supernatant fluid was dialyzed overnight against 0.05 M phosphate buffer (pH 6.7), and used as a crude extract.

Preparation of metallic mercury-releasing enzyme and arabinose dehydrogenase

The crude extract of *Pseudomonas* K62 was fractionated by precipitation with $(NH_4)_2SO_4$ (20 to 70 % saturation), the precipitate was dissolved in 0.05 M phosphate buffer (pH 6.7) and dialyzed against 200 vol. of the same buffer. The dialysate was then applied to a column (2.5 cm $\times$ 90 cm) of Sephadex G-150 which had been equilibrated with 0.05 M phosphate buffer (pH 6.7) and eluted with the same buffer at a flow rate of 10 ml /h Fractions containing metallic mercury-releasing enzyme, arabinose dehydrogenase, cytochrome *c*-I and cytochrome *c*-II were pooled separately. Cytochrome *c*-I was further purified as described below

Assay of metallic mercury-releasing enzyme and arabinose dehydrogenase

Metallic mercury-releasing enzyme was assayed by measuring phenylmercuric acetate decomposition in the following mixture: $5 \cdot 10^{-2}$ M phosphate buffer (pH 5.8) 5 units of arabinose dehydrogenase, $6 \cdot 10^{-5}$ M L-arabinose, $6 \cdot 10^{-5}$ M NADP, $1 \cdot 10^{-7}$ M cytochrome *c*-I, $1 \cdot 10^{-6}$ M FAD, $5 \cdot 10^{-4}$ M thioglycolate, $6 \cdot 10^{-5}$ M phenyl[^{203}Hg]mercuric acetate and the enzyme. The reaction mixture was incubated in a L-form tube (15-cm long, 7-cm high, 2 5-cm diameter) with shaking at 30 °C. Metallic mercury formed from phenylmercuric acetate was rapidly volatilized under this condition, and the radioactivity remaining in the mixture was assayed in a well-type scintillation counter. The activity of arabinose dehydrogenase was measured by reading the increase of absorbance at 340 nm formed by NADPH reduction. The reaction mixture was as follows: $5 \cdot 10^{-2}$ M phosphate buffer (pH 8.5), 3 10^{-3} M L-arabinose, $3 \cdot 10^{-6}$ M NADP, and the enzyme.

Purification and crystallization of cytochrome c-I

The fraction containing cytochrome was separated by chromatography on Sephadex G-150 and concentrated in a collodion bag (Sartorious Membrane Filter Co. Ltd) The enzyme was then applied to a column (2.5 cm × 28 cm) of DEAE-Sephadex A-50 which had previously been equilibrated with 0.05 M phosphate buffer (pH 6.7), and eluted with a linear gradient of 0 to 0.5 M NaCl at a flow rate of 10 ml/h. Fractions containing cytochrome *c*-I were concentrated in a collodion bag, and applied to a column (2.5 cm × 40 cm) of Sephadex G-75 which had been equilibrated with 0.05 M phosphate buffer (pH 6 7). The column was eluted with the same buffer. Crystallization of cytochrome *c*-I was carried out by adding finely powdered $(NH_4)_2SO_4$ to the concentrated enzyme solution. The heme content of cytochrome *c*-I was estimated from the alkaline pyridine ferrohemochrome spectrum[6]. After reduction with a few crystals of dithionite, the absorbance was read at 549 nm Protein contents were estimated as nitrogen content by the micro-Kjeldahl method.

Preparation of bacterial cytochrome c

Cytochromes *c* of bacteria, exept for *Pseudomonas* K62, were separated from each crude extract by chromatography using a column (2.5 cm × 90 cm) of Sephadex G-150. The cytochrome *c* fractions were pooled and stored at −20 °C until use.

Estimation of molecular weight of cytochrome c-I by gel filtration

A Sephadex G-75 column (1.5 cm × 70 cm) was prepared with 0.05 M phosphate buffer (pH 6.7) containing 0.1 M NaCl. Ovalbumin ,α-chymotrypsin and bovine cytochrome *c* were used as an internal standard on the column.

Spectrophotometric measurements

A Hitachi electrophotometer, Type 181, was used for the spectrophotometric measurements.

Chemical

Phenyl[^{203}Hg]mercuric acetate was purchased from the Radiochemical Centre, Amersham, England.

RESULTS

Purification and crystallization of cytochrome c-I

Chromatography of the crude extract on Sephadex G-150 is shown in Fig. 1, indicating the elution of metallic mercury-releasing enzyme, arabinose dehydrogenase, cytochrome *c*-I and cytochrome *c*-II. It has been reported in a previous paper[2] that only the eluted first cytochrome *c* was active for the decomposition of mercurials as an electron carrier and that eluted secondly inactive The active cytochrome was

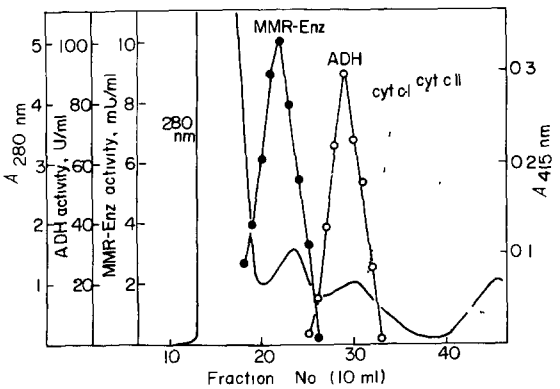


Fig 1 Chromatography of crude extract on Sephadex G-150. A fraction obtained from the crude extract by ammonium sulfate precipitation (0 2–0.7 saturation) was applied to a Sephadex G-150 column (2 5 cm 90 cm), and eluted with 0 05 M phosphate buffer (pH 6 7) Metallic mercury releasing enzyme (MMR-Enz) activity (●–●) and arabinose dehydrogenase (ADH) activity (○–○) was measured for each fraction. Protein (———) was measured by reading absorbance at 280 nm, and cytochromes ( ) by reading at 415 nm

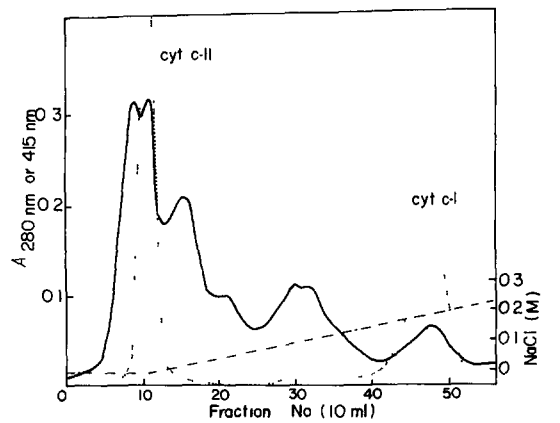


Fig. 2 Chromatography of cytochrome *c*-I and *c*-II on DEAE-Sephadex Fractions of cytochrome *c*-I and *c*-II eluted from the Sephadex G-150 column were pooled, concentrated and then applied to a DEAE-Sephadex A-50 column (2 5 cm 28 cm) which had previously been equilibrated with 0 05 M phosphate buffer (pH 6.7) A linear gradient (– –) of 0 to 0 5 M NaCl in the same buffer was used for elution of cytochromes Protein (———) was measured by reading absorbance at 280 nm, and cytochromes *c* (··) by absorbance at 415 nm.

Fig 3. Crystalline cytochrome *c*-I (300)

designated as cytochrome *c*-I and inactive cytochrome as cytochrome *c*-II. Fractions of cytochrome *c*-I and *c*-II, separated by column chromatography on Sephadex G-150, were further purified by DEAE-Sephadex A-50 chromatography (Fig 2). Cytochrome *c*-I was clearly separated from DEAE-Sephadex chromatography and was then subjected to column chromatography on Sephadex G-75. The main fraction was collected and concentrated for crystallization. To the reddish solution thus obtained, finely powdered $(NH_4)_2SO_4$ was added gradually with gentle stirring until a faint turbidity appeared, and then the solution was kept standing for 3 days at 4 °C. Cytochrome *c*-I was obtained in a crystalline, needle-like form as shown in Fig. 3. The results of a purification experiment are summarized in Table I.

TABLE I

PURIFICATION OF CYTOCHROME *c*-I FROM THE CELL EXTRACT OF *PSEUDOMONAS* K62

Purification step	*Total vol (ml)*	*Total absorbance*		$A_{416\ nm}/A_{280\ nm}$
		280 nm	*416 nm*	
Crude extract	220	16 800	1750	0 104
$(NH_4)_2SO_4$ precipitation	28	11 500	1020	0 089
Sephadex G-150	110	1 360	650	0 478
DEAE-Sephadex A-50	70	66	206	3.20
Sephadex G-75	20	42	150	3.57

Spectral properties of cytochrome c-I

Fig. 4 shows the oxidized and reduced absorption spectra of purified cytochrome *c*-I. The oxidized form shows a Soret band at 407 nm, a δ-band at 360 nm and a diffuse band at 530 nm. When cytochrome *c*-I was reduced with dithionite, the Soret band

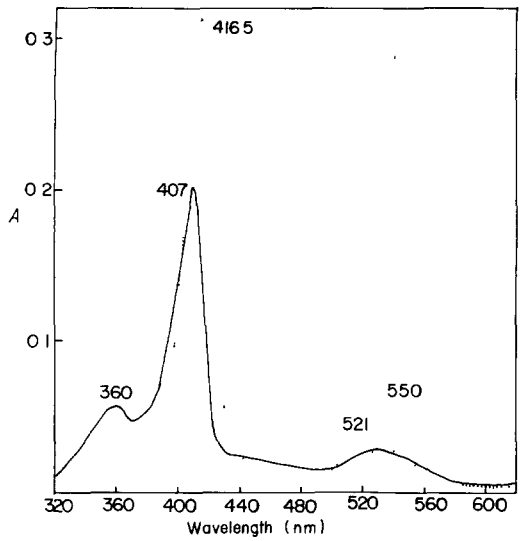


Fig. 4. Absorption spectra of cytochrome *c*-I. (———), oxidized form, ( ), reduced form Oxidation of the cytochrome was carried out by addition of potassium ferricyanide at a final concentration of 0.05 mM For reduction, a small amount of dithionite was added.

peak was intensified and shifted to 416 5 nm, and at the same time α- and β-bands appeared at 550 nm and 521 nm, respectively. The spectral properties of cytochrome *c*-I are summarized in Table II. When the cytochrome was complexed with reduced pyridine ferrohemochrome in alkali, the preparation showed a spectrum with maxima at 549, 520 and 413 nm (Table II). Its spectrum indicates that of a typical *c*-type pyridine ferrohemochrome. Cytochrome *c*-I possesses one heme group per mole of protein.

Effect of cytochrome c-I concentration on the decomposition of phenylmercuric acetate

Double-reciprocal plots of the velocity against phenylmercuric acetate decom-

TABLE II

SPECTRAL PROPERTIES OF K62 CYTOCHROME *c*-I

Absorption maxima (nm)	
Reduced form	550, 521, 416 5
Oxidized form	530, 407, 360
Pyridine ferrohemochrome	549, 520, 413
Extinction ratio	
$A_{416\ 5\ nm\ (red)}/A_{550\ nm\ (oxd)}$	5 57
$A_{521\ nm\ (red)}/A_{407\ nm\ (oxd)}$	0.46
$A_{416\ 5\ nm\ (red)}/A_{407\ nm\ (oxd)}$	1 56
Extinction coefficient ($mM^{-1} \cdot cm^{-1}$)	
550 nm (red)	27.1
521 nm (red)	21.5
416.5 nm (red)	157 8

position and the concentration of cytochrome *c*-I were linear (Fig. 5). The K_m value was calculated to be $0.71 \cdot 10^{-7}$ M.

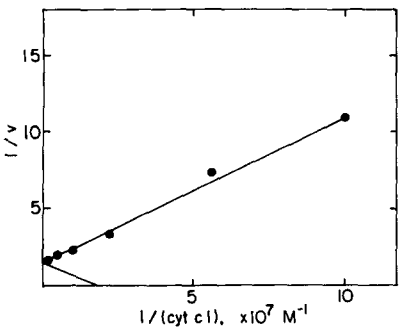


Fig. 5. Relationship between the phenylmercuric acetate decomposition and the concentration of cytochrome *c*-I Initial velocity of phenylmercuric acetate decomposition was measured by following reaction mixture, 5 10^{-2} M phosphate buffer (pH 5.8), 2 munits of metallic mercury releasing-enzyme, 5 units of arabinose dehydrogenase, $6 \cdot 10^{-5}$ M NADP, $3 \cdot 10^{-3}$ M L-arabinose, 1 10^{-6} M FAD, 5 10^{-4} M thioglycolate, 6 10^{-5} M phenyl[^{203}Hg]mercuric acetate, and cytochrome *c*-I.

TABLE III

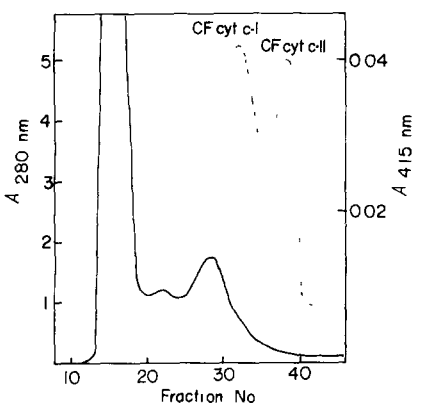
REACTIVITY OF BACTERIAL CYTOCHROME *c* FOR PHENYLMERCURIC ACETATE DECOMPOSITION

Cytochrome *c* was prepared by Sephadex G-150 gel filtration from cell extract of bacteria indicated in the table. The composition of a reaction mixture as follows $5 \cdot 10^{-2}$ M phosphate buffer (pH 5.8), 2 munits metallic mercury-releasing enzyme, 5 units arabinose dehydrogenase, $3 \cdot 10^{-2}$ M L-arabinose, 6 10^{-5} M NADP, $1 \cdot 10^{-6}$ M FAD, $5 \cdot 10^{-4}$ M thioglycolate, $6 \cdot 10^{-6}$ M phenyl(^{203}Hg]-mercuric acetate, and about $5 \cdot 10^{-7}$ M cytochrome *c*.

Cytochrome c	*Phenylmercuric acetate decomposition (μg/ml per h)*
No addition of cytochrome *c*	0
Pseudomonas K62 cytochrome *c*-I	8.4
Pseudomonas K62 cytochrome *c*-II	0
Aeromonas hydrophila (IAM 1018)	0 2
Micrococcus luteus (IAM 1097)	0.3
Bacillus subtilis (IAM 2011)	0.3
Corynebacterium equi (IAM 1038)	0 6
Staphylococcus aureus	0.3
Aerobacter aerogenes (IAM 1019)	0 2
Pseudomonas fluorescens	0.4
Pseudomonas aeruginosa (IAM 2001)	0.2
Pseudomonas ovalis (IAM 1002)	0
Pseudomonas riboflavina	0
Pseudomonas mephitica var. lipolitica	0.1
Pseudomonas CF cytochrome *c*-I	10.9
Pseudomonas CF cytochrome *c*-II	0

Reactivity of cytochromes c prepared from various kinds of bacteria in the phenylmercuric acetate decomposition

Table III shows the effect of various bacterial cytochromes *c* on phenylmercuric acetate decomposition The decomposition system consisted of 5 10^{-2} M phosphate buffer (pH 5.8), 2 munits of metalic mercury releasing enzyme, 5 units of arabinose dehydrogenase, $3 \cdot 10^{-3}$ M L-arabinose, $6 \cdot 10^{-5}$ M phenyl[^{203}Hg]mercuric acetate and about $5 \cdot 10^{-7}$ M cytochrome *c*. As shown in Table III, cytochrome *c* obtained from *Aeromonas hydrophila*, *Micrococcus luteus*, *Bacillus subtilis*, *Corynebacterium equi*, *Staphylococcus aureus*, *Aerobacter aerogenes*, *Pseudomonas fluorescens*, *P. aeruginosa*, *P. ovalis*, *P. riboflavina*, and *P. mephitica var. lipolitica* was inactive at $5 \cdot 10^{-7}$ M in the reaction. On the other hand, cytochrome *c* obtained from *Pseudomonas* CF was found to be capable of coupling the reaction at almost the same rate as cytochrome *c*-I of *Pseudomonas* K62. *Pseudomonas* CF was newly isolated from activated sludge which was acclimatized with $HgCl_2$ and phenylmercuric acetate, and was distinct from *Pseudomonas* K62; for instance, the K62 strain has a red pigment of highly unsaturated carotenoid, but the CF strain is milky white. It was found that *Pseudomonas* CF had two kinds of cytochrome *c* which were separated from each other by chromatography on Sephadex G-150 (Fig. 6). The first eluate, which was designated as CF cytochrome *c*-I, was active for phenylmercuric acetate decomposition. However, the second eluate (CF cytochrome *c*-II) was inactive, as shown in Fig 7. The absorption spectra of CF cytochrome *c*-I and *c*-II in the reduced form are presented in Fig 8.



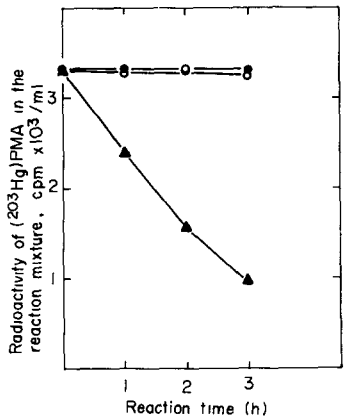


Fig. 6 Gel filtration of the crude extract of *Pseudomonas* CF on Sephadex G-150. The crude extract obtained from the organism was applied to a Sephadex G-150 column (2.5 cm · 90 cm), and eluted with the same buffer (pH 6 7). Protein was measured by reading absorbance at 280 nm (———), and cytochromes by absorbance at 415 nm ( ).

Fig. 7 Reactivity of CF cytochrome *c*-I and *c*-II for the phenylmercuric acetate (PMA) decomposition The reactivity of fraction No 32 and No. 36 obtained from Sephadex G-150 eluate (Fig 6) was examined for the phenylmercuric acetate decomposition. The composition of a reaction mixture was as follows $5 \cdot 10^{-2}$ M phosphate buffer (pH 5 8), 2 munits of metallic mercury releasing enzyme 5 units of arabinose dehydrogenase, 6 10^{-5} M NADP, 3 10^{-3} M L-arabinose, $1 \cdot 10^{-6}$ M FAD, 5 10^{-4} M thioglycolate, $6 \cdot 10^{-5}$ M phenyl[^{203}Hg]mercuric acetate, and fraction No. 32 or No 36 (▲–▲), No 32 fraction was added; (○–○), No. 36 fraction was added, (●–●), no addition

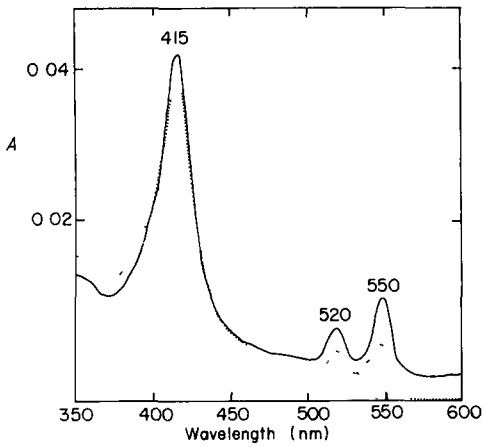


Fig 8 Absorption spectra of CF cytochrome *c*-I and *c*-II (———), CF cytochrome *c*-I (reduced form), ( ), CF cytochrome *c*-II (reduced form) The reduction of cytochrome *c* was carried out by addition of a small amount of dithionite.

Molecular weight of CF cytochrome c-I

The molecular weight of CF cytochrome *c*-I was estimated by gel filtration using Sephadex G-75 (Fig. 9). Judging from the elution profile of CF cytochrome *c*-I, the molecular weight was estimated to be about 26 000, which is approximately equivalent to the molecular weight of K62 cytochrome *c*-I

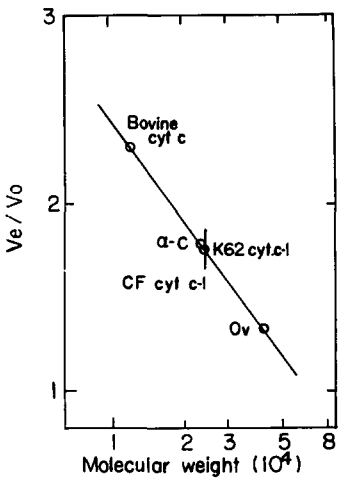


Fig. 9 Estimation of molecular weight of CF cytochrome *c*-I by gel filtration A Sephadex G-75 column (1 5 cm 70 cm) equilibrated with 0.05 M phosphate buffer (pH 6.7) containing 0 1 M NaCl was used The column was calibrated with respect to molecular weight with ovalbumin (Ov) 45 000, α-chymotrypsin (α-C) 25 000, bovine cytochrome *c* (Bovine cyt. *c*) 12 400 and K62 cytochrome *c*-I (K62 cyt *c*-I) 26 000[2] Dextran blue was used as a void volume marker. Eluted protein was determined by absorbance at 280 nm Cytochromes were determined by absorbance at 415 nm.

DISCUSSION

It is known that a *c*-type cytochrome is involved in the reduction of nitrate, nitrite, sulfate and sulfite as an electron carrier in anaerobic respiration. Cytochrome *c*(551) acted as an electron carrier of the nitrite reductase system in *Pseudomonas aeruginosa*[7]. Ishimoto *et al.*[8] and Postgate[9,10] suggested that cytochrome c_3 was concerned in the sulfate reduction. However, it has not been reported so far that the *c*-type of cytochrome is involved in the reduction of mercurials. In previous papers[1-4], we described the mechanism of enzymatic decomposition of organomercurials to metallic mercury in mercury-resistant *Pseudomonas* K62. The reductive reaction was considered to be as follows: NAD(P)H is generated by glucose dehydrogenase or arabinose dehydrogenase system, and the electrons are transferred to metallic mercury-releasing enzyme, which has FAD as a prosthetic group, in the presence of cytochrome *c*-I. Organomercurials would become a terminal acceptor of electrons, and then be reduced to metallic mercury by an cooperative action of metallic mercury-releasing enzyme and cytochrome *c*-I.

Cytochrome *c*-I was confirmed to be a *c*-type cytochrome. Yamanaka and Okunuki[11] indicated that there were two functionally distinct types of cytochrome *c*: those from plants and animals react with cytochrome oxidase derived from mammalian sources. and those from bacteria react with cytochromes oxidase derived from bacterial sources. However, bacterial cytochromes *c* obtained from type cultures belonging to the genera *Aeromonas*, *Micrococcus*, *Bacillus*, *Corynebacterium*, *Staphylococcus*, *Aerobacter*, and *Pseudomonas* were all inactive at the concentration tested in the phenylmercuric acetate decomposing system of *Pseudomonas* K62. On the other hand, cytochrome *c* obtained from *Pseudomonas* CF (CF cytochrome *c*-I) was capable of coupling in the reaction at almost the same rate as K62 cytochrome *c*-I *Pseudomonas* CF was isolated from the activated sludge acclimatized with $HgCl_2$ and phenylmercuric acetate, and was quite different from *Pseudomonas* K62 with respect to taxonomic properties. It is interesting that the CF strain as well as the K62 strain had two kinds of cytochrome *c* which are different in molecular weight and function, CF cytochrome *c*-I and K62 cytochrome *c*-I had the same mol. wt of 26 000 and were active in phenylmercuric acetate decomposition. Furthermore, CF cytochrome *c*-II which had a low molecular weight was inactive in the reaction. Purification of CF cytochrome *c*-I and enzymatic decomposition of mercurials in CF strain are now in progress. It is likely that the decomposition mechanism found in the K62 strain will also exist in the CF strain.

Some cytochromes whose function was obscure were reported. Cytochrome c_{556}, a diheme protein obtained from *Pseudomonas aeruginosa*, has a mol. wt of 77 200, but the function was not clear; it did not react with cytochrome oxidase of the organism[12]. The relationship between cytochrome *c*-I and cytochrome *c*-II, nor their involvement in the respiratory chain have as yet been disclosed. The formation of metallic mercury-releasing enzyme was specifically induced in the presence of mercurials, but the formation of cytochrome *c*-I was not[4]. Therefore, it may not be said that cytochrome *c*-I serves only for the mercurial reduction. It would be very interesting to know how widely cytochromes such as K62 cytochrome *c*-I or CF cytochrome *c*-I which are involved in the decomposition of organomercurials are distributed in other microorganisms and serve for the mercurial reduction.

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