Biochimica et Biophysica Acta, 522 (1978) 291—302

BBA 68349

METHYLAMINE DEHYDROGENASE OF *PSEUDOMONAS* sp. J

PURIFICATION AND PROPERTIES

TAKASHI MATSUMOTO

Department of Chemistry, College of Science, Rikkyo (St. Paul's) University, Nishi-Ikebukuro, Toshima-ku, Tokyo, 171 (Japan)

(Received June 20th, 1977)

Summary

Methylamine dehydrogenase was purified in a homogeneous form from methylamine-grown *Pseudomonas* sp. J. The specific activity of the purified enzyme was 5.19 at 19°C. The molecular weight was estimated to be 105 000, and the enzyme was composed of two kinds of subunit with molecular weights of 40 000 and 13 000, respectively. The enzyme contained little phosphorus, iron and copper. The enzyme had absorption maxima at 278, 330, 430 and 460 nm (shoulder). On addition of methylamine, the peaks at 430 and 460 nm decreased, while that at 330 nm increased. Primary amines served as substrates, but secondary and tertiary amines did not. Phenazine methosulfate was the most effective electron acceptor and oxygen was ineffective. The enzyme was inhibited by carbonyl reagents, cuprizone and $HgCl_2$ but not by other chelators or sulfhydryl reagents. Some of other physical and biochemical properties of the enzyme were studied. These results show that the enzyme purified from *Pseudomonas* sp. J is essentially similar to the enzyme obtained from *Pseudomonas* AM1, although it differs slightly in some properties.

Introduction

Eady and Large [1] first reported that methylamine dehydrogenase purified from *Pseudomonas* AM1 oxidizes methylamine to formaldehyde and ammonia in the presence of phenazine methosulfate as an electron acceptor. Among enzymes oxidizing amines, this enzyme has unique properties, especially with regard to its prosthetic group. They did not identify the prosthetic group, but they assumed that it was a derivative of pyridoxal [2]. The natural electron acceptor of the enzyme has also not yet been characterized.

We isolated a soil bacterium, identified as *Pseudomonas* sp. J, which can grow on C-1 compounds such as methylamine and methanol as a sole carbon

source. A methylamine dehydrogenase similar to that of *Pseudomonas* AM1 was induced in the bacterium when it was grown on methylamine as a sole carbon source.

This paper reports the purification of this enzyme and a comparison of its physicochemical and catalytic properties with those of the *Pseudomonas* AM1 enzyme.

Materials and Methods

Chemicals. The chemicals used were obtained as follows: carboxymethyl (CM)-cellulose from Brown Co.; Sephadex G-150 and G-200 from Pharmacia Fine Chemicals; ovalbumin (Grade III), catalase (C-100) (EC 1.11.1.6), and glyceraldehyde-3-phosphate dehydrogenase (rabbit muscle) (EC 1.2.1.12) from Sigma Chemical Co.; trypsin inhibitor (soybean), cytochrome *c* (horse heart), and alcohol dehydrogenase (yeast) (EC 1.1.1.1) from Boehringer Mannheim; bovine serum albumin from Kokusan Chemical Works; NAD^+ and $NADP^+$ from Oriental Yeast Co.; FAD and FMN from Kyowa Hakko Co.; phenazine methosulfate (PMS) from Nakarai Chemicals Co.; and Ampholine (pH 3.5—10.0) from LKB-Producter. All other reagents used were reagent grade materials.

Bacterium and growth. This organism was isolated from garden soil by repeated selection of single colonies from agar plate containing 0.5% methylamine hydrochloride and inorganic salts [3]. The features of the bacterium were as follows: punctiform to circular, convex, entire, smooth, glistening, white and translucent or opalescent on methylamine and salt agar plates; straight rods occurring singly or in pairs, motile with a single polar flagellum, no spore formation and gram-negative; only methylamine and methanol were assimilated as a carbon source. The following compounds were not assimilated: dimethylamine, putrescine, ethanol, formate, acetate, propionate, citrate, succinate, malate, glycine, serine, glucose, fructose and bouillon. From these results, we have tentatively named this obligate methylotrophic bacterium *Pseudomonas* sp. J.

The bacterial cells were grown at 28°C with aeration in the liquid medium [3], and were harvested when the absorbance at 650 nm reached 0.7—0.8 (after about 40 h). The packed cells were kept at −20°C.

Standard enzyme assay. The method for enzyme assay was essentially the same as described by Eady and Large [1]. Cuvettes (1 cm light path) contained in a total volume of 1 ml: 100 μmol of sodium phosphate buffer, pH 7.5, 0.33 μmol of phenazine methosulfate, 0.043 μmol of 2,6-dichlorophenolindophenol (DCPIP), and 6.7 μmol of methylamine hydrochloride. The reaction was started by the addition of enzyme. The rate of reduction of DCPIP in the reaction mixture was measured with a Hitachi 124 spectrophotometer at room temperature. The specific activity was expressed as units per mg of protein. One unit of enzyme was defined as the amount reducing 1 μmol of DCPIP per min under the above conditions.

Chemical determination. Either the biuret method [4] or the method of Lowry et al. [5] was employed for the determination of protein concentration, using bovine serum albumin as a standard. Total phosphorus was determined by the method of Ames [6].

Optical measurements. A Union model SM-401 recording spectrophotometer was used to measure the absorption spectrum, a Hitachi 204 spectrofluorimeter for recording the fluorescence spectrum and a JASCO J-20 automatic recording spectropolarimeter for recording the circular dichroism spectrum at room temperature. Copper and iron were determined by atomic absorption spectrophotometry using a Varian Techtron 63 carbon rod atomizer with a Nippon Jarrell-Ash flameless atomizer FLA-10.

Electrophoresis. Polyacrylamide gel electrophoresis was performed by the method of Reisfeld et al. [7]. Protein was stained with Amido Black 10B and material with enzyme activity was stained with a solution containing (per ml); sodium phosphate buffer, pH 7.5, 100 μmol; methylamine hydrochloride, 15 μmol; nitroblue tetrazolium, 500 μg and phenazine methosulfate, 50 μg. SDS-polyacrylamide gel electrophoresis was carried out by the method of Weber and Osborn [8]. Ampholine, pH 3.5—10 and an 8100-1 LKB column were used for determining the isoelectric point, following the method of Vesterberg [9]. A Toa Dempa HM-5A pH meter equipped with a GC-195 combination electrode was used to measure the pH at 15°C.

Amino acid analysis. A JEOL JLC-6AS amino acid analyzer with a single column was used. Half-cystine was determined as cysteic acid after performic acid oxidation [10]. Tryptophan was analyzed by the method of Matsubara and Sasaki [11].

Molecular weight. Molecular weight was determined by the method of Andrews [12] using trypsin inhibitor (21 500), ovalbumin (45 000), bovine serum albumin (67 000), glyceraldehyde-3-phosphate dehydrogenase (145 000), alcohol dehydrogenase (150 000), and catalase (240 000) as standards.

Results

Purification of methylamine dehydrogenase. All the procedures were carried out at 0—7°C.

Cells (126 g wet weight) were thawed and suspended with 10 mM potassium phosphate buffer, pH 7.5. The homogenate was sonificated and centrifuged at 14 000 × *g* for 30 min. The supernatant, designated as crude extract, was fractionated by addition of solid $(NH_4)_2SO_4$. Material precipitated with between 45 and 95% saturation of $(NH_4)_2SO_4$ was collected by centrifugation and dialyzed against 50 mM phosphate buffer, pH 5.2. The dialyzed solution was applied to a CM-cellulose column (2.5 × 25 cm) equilibrated with the same buffer, pH 5.2. The column was washed with the buffer and then the enzyme was eluted with linear gradient of NaCl (0—0.25 M) in the buffer, pH 5.2. Fractions with activity were collected and concentrated in a collodion bag. The concentrated solution was passed through a Sephadex G-150 column (3.0 × 42.5 cm) equilibrated with 10 mM phosphate buffer, pH 7.0. Then the enzyme active solution was applied to a column (1.5 × 21 cm) of hydroxyapatite equilibrated with 10 mM potassium phosphate buffer, pH 7.0. The protein was eluted with a linear gradient (0.01—1.0 M) of the same buffer, pH 7.0. Fractions with activity were combined and concentrated in a collodion bag. The results of the purification are summarized in Table I.

TABLE I

PURIFICATION OF METHYLAMINE DEHYDROGENASE FROM *PSEUDOMONAS* sp. J

Methylamine dehydrogenase activity was estimated at 19°C.

Step	Volume (ml)	Protein (mg)	Total units	Specific activity (units/mg of protein)
Crude extract	500	5488	1395	0.25
Ammonium sulfate fractionation (45—95%)	82	2225	1057	0.48
CM-cellulose chromatography	160	179.4	803.7	4.48
Sephadex G-150 chromatography	39	160.2	825.3	5.15
Hydroxyapatite chromatography	48	128.1	665.3	5.19

The purified enzyme solution was greenish-yellow and specific activity of the enzyme was 5.19 at 19°C. The enzyme was stable at −20°C for several months.

Homogeneity of the enzyme. The purified enzyme appeared homogeneous on polyacrylamide gel electrophoresis (Fig. 1). The enzyme gave a single band of protein on the gel, which coincided with the single band of enzyme activity.

Molecular weight. The molecular weight of the enzyme was calculated to be 105 000 by the gel filtration method (Fig. 2). Thus the molecular weight of the *Pseudomonas* sp. J enzyme is lower than 133 000 of the *Pseudomonas* AM1 enzyme [2].

SDS-polyacrylamide gel electrophoresis. The enzyme was treated with 8 M urea and subjected to SDS-polyacrylamide gel electrophoresis. Two bands were observed (Fig. 3). The molecular weights of the faint, faster moving band and the densely stained, slowly migrating band were calculated to be 13 000 and 40 000, respectively (Fig. 4). The results indicate that the enzyme consists of two subunits differing in molecular weight.

Isoelectric point. The isoelectric point of the enzyme was determined to be pH 9.0 by isoelectric focusing. The enzyme was a basic protein, as expected from the results of CM-cellulose column chromatography and polyacrylamide gel electrophoresis.

Amino acid composition. Table II shows the amino acid composition of the enzyme: numbers of residues are shown as nearest integers. Samples were hydrolyzed directly with 6 M HCl because no prosthetic group was liberated from the enzyme on heat or acid treatment in the presence or absence of methylamine. No free sulfhydryl groups could be detected in the native or heat-treated enzyme by the method of Boyer [13]. The content of polar amino acids of the enzyme protein was calculated to be 46% from Table II [14]. This value is typical for a soluble enzyme.

Phosphorus, iron, and copper contents. The enzyme solution was first dialyzed against 2000 volumes of 0.1 M NaCl and then the enzyme was dialyzed against distilled water. This dialyzed enzyme was fully active and contained little phosphorus, iron, and copper (0.001, 0.006, and 0.007 g atoms per mol of enzyme).

pH and thermal stabilities. The enzyme was incubated at pH values of 3.0—10.6 at 30°C for 75 min. No activity was lost on incubation at above pH

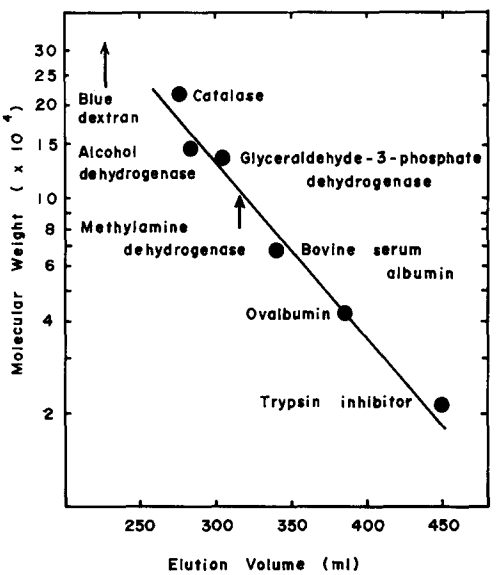
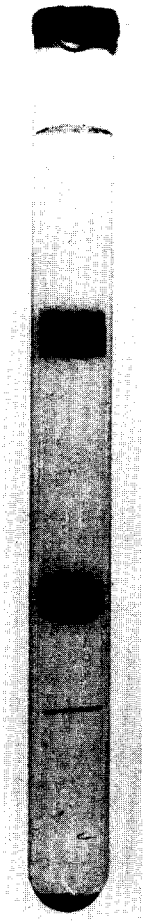


Fig. 1. Polyacrylamide gel electrophoresis of the purified methylamine dehydrogenase. Approx. 30 μg of protein was applied to 7.5% polyacrylamide gel and electrophoresis was carried out for 4 h at 2 mA per gel. After electrophoresis, the left-hand gel was stained with Amido Black 10B for protein and the right-hand gel was stained for enzyme activity by the method described in Materials and Methods.

Fig. 2. Estimation of the molecular weight of methylamine dehydrogenase by Sephadex G-200 gel chromatography. A column (3.8 × 53 cm) of Sephadex G-200 was equilibrated with 50 mM Tris · HCl buffer, pH 7.4, containing 0.1 M KCl. The void volume was 228 ml and the elution volume of the purified enzyme was 316 ml.

4.4. Moreover, no activity was lost on heating at 55°C for 15 min, although 70% of the activity was lost on heating at 70°C for 15 min at pH 7.0. These results show that the enzyme is less stable than the *Pseudomonas* AM1 enzyme to both pH and heat treatments (Figs. 5 and 6).

Optical properties. The absorption spectra of the enzyme are shown in Fig. 7. The enzyme had absorption maxima at 278, 330, 430 and 460 nm (shoulder). The peak at 330 nm increased on addition of methylamine, while those at around 430 and 460 nm decreased, leaving a small peak at 425 nm. The spectral change produced by addition of methylamine was retained after



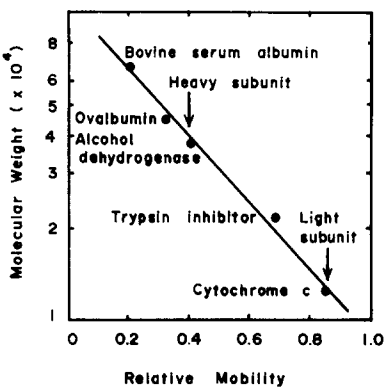


Fig. 3. SDS-polyacrylamide gel electrophoresis of methylamine dehydrogenase. The enzyme was treated with 8 M urea containing 1% SDS, 1% 2-mercaptoethanol, and 10 mM phosphate buffer, pH 7.0 at room temperature for 12 h. Then this mixture was dialyzed against 10 mM sodium phosphate buffer, pH 7.0, containing 0.1% SDS and 0.1% 2-mercaptoethanol. Electrophoresis was performed at 8 mA per gel for 4.5 h using 10% polyacrylamide gel and about 60 μg of enzyme protein.

Fig. 4. Estimation of the molecular weights of the subunits of methylamine dehydrogenase. The conditions were the same as in Fig. 3, except that 20 μg of the enzyme was used. Electrophoretic mobilities of polypeptides are plotted against the logarithms of their molecular weights: bovine serum albumin (67 000), ovalbumin (43 000), alcohol dehydrogenase (38 000), trypsin inhibitor (21 500), and cytochrome *c* (12 400).

dialysis against 50 mM phosphate buffer. The addition of PMS to this modified enzyme restored the spectrum of native enzyme. The spectrum was very similar to that of the *Pseudomonas* AM1 enzyme. On excitation at 288 nm a fluorescence spectrum with maxima at 333 and 370 nm was observed. On excitation at 330 nm, the peak of emission was at 380 nm and this was either increased or stabilized by the addition of methylamine. Circular dichroism spectrum was shown in Fig. 8. The spectral data are expressed in terms of mean residue ellipticity, $[\theta]$. Mean residue molecular weight was calculated to be 110.1

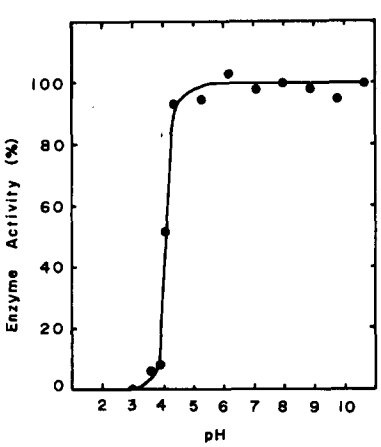
TABLE II

AMINO ACID COMPOSITION OF METHYLAMINE DEHYDROGENASE

Each value is the average and is expressed to the nearest integer. Values for serine and threonine were determined by extrapolation to zero time. The values for valine and isoleucine were those of 72 h hydrolysis.

Amino acid	Residues per 105 000 g of methylamine dehydrogenase
Lys	69
His	21
Arg	40
Trp	9
Asx	105
Thr	56
Ser	70
Glx	87
Pro	62
Gly	77
Ala	68
Cys	27
Val	66
Met	21
Ile	34
Leu	80
Tyr	34
Phe	45

from the amino acid composition. The α-helix content of the enzyme was calculated to be less than 1% by the method of Greenfield and Fasman [15]. The mean residue ellipticities of the enzyme were smaller than those of other



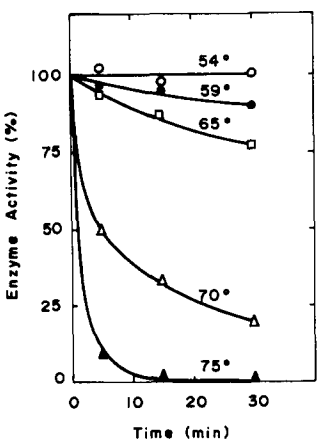


Fig. 5. pH stability of the enzyme. The enzyme (0.15 mg/ml) was incubated at 30°C for 75 min in 0.25 M of the buffer at the pH indicated in the figure. The buffers used in this experiment were glycine/HCl buffer (pH 3.0—4.0), sodium acetate buffer (pH 4.0—5.5), potassium phosphate buffer (pH 5.5—8.5), and glycine/NaOH buffer (pH 8.5—10.7). Samples of 40 μl were used in the assay.

Fig. 6. Thermal stability of the enzyme. The enzyme (0.38 mg/ml) was incubated in 50 mM sodium/potassium phosphate buffer, pH 7.0, at the temperature indicated in the figure. Samples of 20 μl were assayed at the various times indicated.

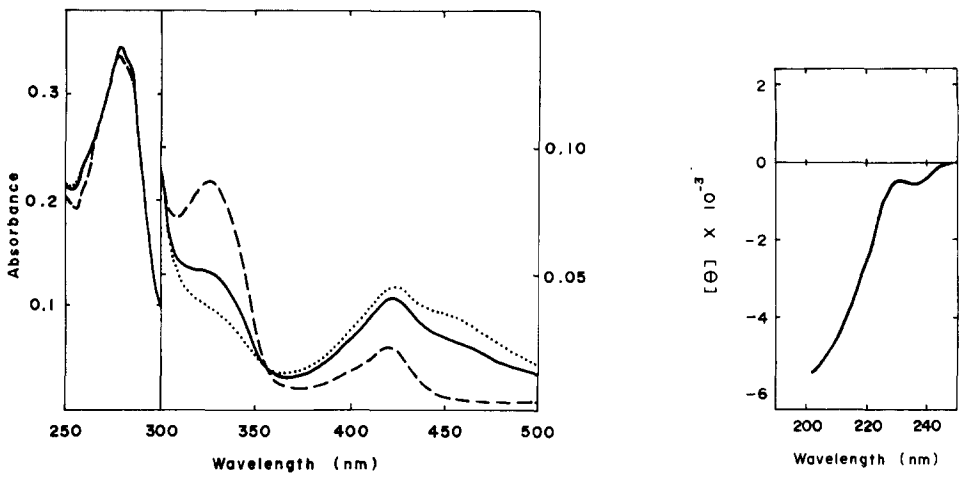


Fig. 7. Absorption spectra of methylamine dehydrogenase. The cuvette contained 0.75 mg of the purified enzyme in 2 ml of 10 mM potassium phosphate buffer, pH 7.0: (a) ———, purified enzyme; (b) - - - - - -, purified enzyme plus methylamine hydrochloride (2 μM); and (c) · · · · · ·, 1 μM phenazine methosulfate added to b.

Fig. 8. Circular dichroism spectrum of methylamine dehydrogenase. The enzyme (0.6 mg/ml of 50 mM potassium phosphate buffer, pH 7.0) was measured in a cell of 0.63 mm light-path length at 20°C.

known proteins, and the spectrum was similar to that of random coil.

pH optimum and effect of temperature. The optimal pH of the reaction was 7.5 in phosphate buffer. Half-maximal activities were observed at pH 6.5 and 9.0, respectively. The enzyme reaction was temperature dependent (Fig. 9).

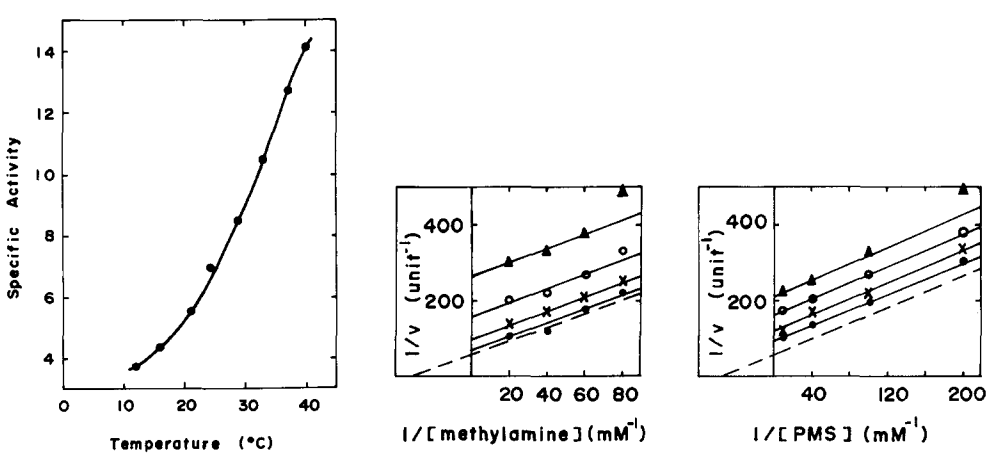


Fig. 9. Effect of assay temperature on the rate of enzyme activity. The rate of enzyme activity was determined at the indicated temperatures in the standard assay conditions.

Fig. 10. Kinetics of the reaction of methylamine dehydrogenase. Left: double reciprocal values of the velocity of the enzyme reaction plotted against the methylamine concentration at four fixed concentrations of PMS (▲———▲, 5 μM; ○———○, 10 μM; X———X, 25 μM; and ●———●, 100 μM). The dashed line indicates secondary plots of the intercepts ($1/V$) of the right-hand figure. Right: double reciprocal values of the velocity of the enzyme reaction plotted against the PMS concentration at four fixed concentrations of methylamine (▲———▲, 12.5 μM; ○———○, 16,7 μM; X———X, 25 μM; and ●———●, 50 μM). The dashed line indicates secondary plots of the intercepts ($1/V$) of the left-hand figure.

TABLE III

SUBSTRATE SPECIFICITY OF METHYLAMINE DEHYDROGENASE

Reactions were measured spectrophotometrically under assay conditions except that methylamine hydrochloride was replaced by other compounds as indicated. All substrates were added to a concentration of 10 mM.

Substrate	Relative reduction rate (%)
Methylamine	(100)
Ethylamine	36.9
n-Propylamine	16.9
n-Butylamine	9.0
Dimethylamine	0
Trimethylamine	0
Tetramethylammonium chloride	0
Benzylamine	0
1,3-Diaminopropane	15.9
1,4-Diaminobutane	9.1
1,6-Diaminohexane	7.6
Spermidine	2.7
Spermine	1.6
Alanine	0
β-Alanine	0
Glycine	0
Serine	0
Lysine	0
Ornithine	0
Ethanolamine	31.7

TABLE IV

SPECIFICITY OF METHYLAMINE DEHYDROGENASE FOR ELECTRON ACCEPTORS

All reactions except that with oxygen as an electron acceptor were measured spectrophotometrically at the various wavelengths indicated in the table. Oxygen consumption was measured in a Beckman Oxygen Analyzer, model 777. The reaction mixtures contained (in μmol) : sodium phosphate, pH 7.5, 100; methylamine hydrochloride, 6.7; and electron acceptor as indicated in the table. Experiments with FAD and FMN as electron acceptors were carried out under anaerobic conditions in a Thunberg-type cuvette.

Acceptor	Final concentration (mM)	Wavelength (nm)	Relative activity (%)
PMS	0.33		
plus DCPIP	0.043	600	(100)
plus ferricyanide	1.0	420	253
plus cytochrome *c*	0.05	550	247
DCPIP	0.043	600	3.95
Ferricyanide	1.0	420	21.5
Cytochrome *c*	0.05	550	1.80
NAD^+	0.1	340	0
$NADP^+$	0.1	340	0
FAD	0.05	450	0
FMN	0.05	450	0
Vitamin K-3	0.95		
plus DCPIP	0.043	600	0
Oxygen			0

The activation energy of the reaction was calculated to be 8.8 kcal/mol by the Arrhenius plots.

Substrate specificity. Primary amines served as substrates for the enzyme but a secondary amine, dimethylamine, and a tertiary amine, trimethylamine, did not, as shown in Table III. Diamines and polyamines were less good substrates than methylamine. Furthermore, ethanolamine was oxidized at the same rate as ethylamine.

Michaelis constant. Michaelis constants of 30 μM for methylamine and 18 μM for PMS were calculated from the velocities versus the concentration of methylamine and phenazine methosulfate (Fig. 10). The secondary plots indicate that the reaction proceeds via a ping-pong mechanism, like that reported the *Pseudomonas* AM1 enzyme [2].

Electron acceptors. Among the possible electron acceptors tested, phenazine methosulfate was most effective for the enzyme reaction; ferricyanide and cytochrome *c* were less effective. NAD^+, $NADP^+$, FAD, FMN, vitamin K-3 and oxygen did not act as electron acceptors (Table IV).

Effects of carbonyl reagents and other compounds. Semicarbazide, isoniazide, $HgCl_2$ and cuprizone inhibited the enzyme (Table V). However, metal chelators and sulfhydryl reagents were not inhibitory. These results suggest that the active site of the enzyme may contain a carbonyl group.

TABLE V

EFFECTS OF INHIBITORS ON METHYLAMINE DEHYDROGENASE ACTIVITY

Enzyme activities were measured spectrophotometrically. The reaction mixture contained (in μmol): sodium phosphate buffer, pH 7.5, 100; DCPIP, 0.043; PMS, 0.124; enzyme (6 μg); and inhibitor (total volume, 0.9 ml). After incubation of the mixtures in the dark for 30 min at 30°C, the reactions were started by the addition of 10 mM methylamine hydrochloride.

Inhibitor	Final concentration (mM)	Inhibition (%)
None		(0)
KCN	1	0
NaN_3	1	0
α,α'-Bipyridyl	1	0
8-Hydroxyquinoline	1	0
Ethylenediaminetetraacetic acid disodium salt	1	0
Cuprizone	0.1	100
	0.01	100
	0.001	28.7
o-Phenanthroline	1	0
5,5'-Dithiobis (2-nitrobenzoic acid)	1	0
Monoiodoacetate	1	0
p-Chloromercuribenzoate	0.5	0
$HgCl_2$	1	100
	0.01	23
Semicarbazide	0.1	100
	0.01	100
	0.001	59.2
Isoniazide	1	95.0
	0.01	15.6

Discussion

Pseudomonas sp. J forms colorless colonies on agar plates and is thought to be an obligate methylotroph from the carbon source utilizations. The bacterium is different from so-called red methanol utilizers, such as *Pseudomonas* AM1 [16] and *Pseudomonas* M27 [17], but very similar to *Pseudomonas* strain W1 [18,19] and methanol-utilizing bacteria classified as group IIc by Kouno and Ozaki [20]. The presence of a phenazine methosulfate-linked methylamine dehydrogenase in cells of *Pseudomonas* strain W1 grown on methylamine was reported by Dahl et al. [18].

The enzyme was formed inducibly in the methylamine-grown cells but was not found in cells assimilating methanol as a carbon source. The enzyme protein constituted about 5% of the total protein of the crude extract.

The molecular weight of 105 000 is smaller than that of 133 000 for the *Pseudomonas* AM1 enzyme [2]. The enzyme was found to consist of two kinds of subunit and suggested to be $\alpha_2\beta_2$-type subunit structure from the molecular weights of the enzyme and subunits. The enzyme is less stable at extreme pH and on heating than the *Pseudomonas* AM1 enzyme, and this might be related to the difference in its molecular structure.

The *Pseudomonas* AM1 enzyme has been suggested to have a derivative of pyridoxal as a prosthetic group on the basis of its absorption spectrum, inhibition by carbonyl reagents and the chromatographic behavior of the isolated prosthetic group [2]. The *Pseudomonas* sp. J enzyme has a similar absorption spectrum and susceptibility to carbonyl reagents as the *Pseudomonas* AM1 enzyme, but unlike the latter, the prosthetic group could not be resolved from the enzyme even by heat treatment at 100°C for 15 min in the presence of methylamine. Thus, the prosthetic group seems to be more tightly bound to this enzyme than to the *Pseudomonas* AM1 enzyme.

It has been reported that it is very difficult to isolate the prosthetic group from monoamine oxidase of beef plasma [21] and *Aspergillus niger* [22], diamine oxidase of pea seedlings [23], and trimethylamine dehydrogenase of obligate methylotroph Bacterium W3A1 [24,25]. Monoamine and diamine oxidases were reported to contain derivatives of pyridoxal phosphate and copper as prosthetic groups, and trimethylamine dehydrogenase was to contain non-heme iron and acid-labile sulfide, and covalently bound, phosphorylated chromophore assumed to be pteridine derivative. The enzyme examined in this work contained only minute amounts of copper, iron and phosphorus and was not inhibited by metal chelators. Therefore, it seems unlikely that copper or iron participates in the enzyme reaction.

Anthony [26] suggested that cytochrome *c*-551 is essential part of electron transport system of methanol and methylamine oxidation in the primary electron acceptor of methanol dehydrogenases of *Pseudomonas* AM1. Like methylamine dehydrogenase, methanol dehydrogenase can also utilize phenazine methosulfate as an electron acceptor. In a preliminary experiment, we found that a cytochrome *c*-551 from *Pseudomonas* sp. J could serve as a primary electron acceptor. The rate of reduction of cytochrome *c*-551 was the same as that of horse heart cytochrome *c* and was about 2% of that of PMS (unpublished data). If a cytochrome is involved, then this result suggests that there is an

intermediary acceptors between it and the enzyme.

The enzyme was relatively resistant to sulfhydryl reagents and no sulfhydryl groups could be detected. The observed inhibition by $HgCl_2$ was thus probably not due to its action on sulfhydryl groups.

After this and subsequent manuscripts had been completed, Mehta [27] reported purification and properties of a methylamine dehydrogenase from *Methylomonas methylovora*, an obligate methylotroph. The properties of the enzyme resemble to those of *Pseudomonas* sp. J in heat stability, pH optimum and effect of inhibitor except for *p*-chloromercuribenzoate inhibition, but differ in specific activity, specificities for substrate and electron acceptor. The physicochemical properties and subunit structure of the enzyme were not described.

Acknowledgements

The author wishes to express his gratitude to Dr. J. Tobari for many helpful suggestions and discussions and to Miss J. Takeuchi for her technical assistance. He is also grateful to Dr. K. Kouno of Sanraku Ocean Co. for identification of the bacterium, Mr. Y. Motoki and Dr. I Muramatsu of this university for amino acid analysis, and Drs. Y. Takada and K. Nakano of this university for analyses of copper and iron.

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