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Trimethylamine Oxide Respiration in *Proteus* sp. Strain NTHC153: Electron Transfer-Dependent Phosphorylation and L-Serine Transport

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Cells of *Proteus* sp. strain NTHC153 grown anaerobically with glucose and trimethylamine oxide (TMAO) were converted to spheroplasts by the penicillin method. The spheroplasts were lysed by osmotic shock, and the membrane vesicles were purified by sucrose gradient centrifugation. Vesicles energized by electron transfer from formate to TMAO displayed active anaerobic transport of serine. An anaerobic cell-free extract of *Proteus* sp. disrupted in a French pressure cell reduced TMAO with formate and NADH with the concomitant formation of organic phosphate. The net P/2e$^-$ ratios determined were 0.1 and 0.3, respectively. The NADH- and TMAO-dependent phosphorylation was sensitive to uncouplers of oxidative phosphorylation (protonophores), and the formate- and TMAO-dependent serine transport was sensitive to ionophores and protonophores. We conclude that TMAO reduction in *Proteus* sp. fulfills the essential features of anaerobic respiration.

Respiratory systems in bacteria and mitochondria are characterized by the generation of a proton motive force and the synthesis of ATP catalyzed by membrane-bound ATPases. According to the chemiosmotic hypothesis, ATP synthesis is driven by the proton motive force which consists of a transmembrane pH gradient and an electrical potential. In bacteria the proton motive force, or its components, provokes active transport of amino acids and certain other metabolites (for review, see references 3, 7, and 11).

The best-known examples of anaerobic respiration in species of *Enterobacteriaceae* are the so-called nitrate and fumarate respirations. The mechanism of energy conservation in these processes has been studied extensively in *Escherichia coli* by use of various in vitro experimental techniques including determination of ATP synthesis in subcellular preparations (23–26) and active transport of amino acids and lactose in membrane vesicles (1, 17–19).

There exists a less familiar type of anaerobic respiration in enterobacteria: namely, trimethylamine oxide (TMAO) respiration. TMAO, which is a major low-molecular-weight constituent of most marine animals (8, 30), stimulates the anaerobic growth of *E. coli* (13, 34), *Salmonella typhimurium* (16), and *Proteus* sp. strain NTHC153 (32). These enterobacteria reduce TMAO to trimethylamine (TMA), and the growth data suggest that the reduction of TMAO is coupled with conservation of energy by a respiratory mechanism. This is supported by the finding of an electron transfer chain to TMAO in *E. coli* involving *b*- and *c*-type cytochromes (28), and an energizing of the cytoplasmic membrane of *E. coli* by NADH and TMAO has been shown by determination of electron transfer-dependent quenching of atebrin fluorescence (5). In the present paper we report that electron transfer to TMAO sustains anaerobic serine transport in membrane vesicles and phosphorylation in cell-free extract of *Proteus* sp. strain NTHC153 prepared in a French pressure cell.

MATERIALS AND METHODS

Bacterial strain and growth conditions. *Proteus* sp. strain NTHC153, originally isolated from a fish gill (31), was grown in a tryptone-yeast extract basal medium (32) supplemented with 1 μM sodium molybdate, 1 μM selenic oxide, 10 mM glucose, and 30 mM TMAO. The growth medium (1 liter) in a bottle with a rubber stopper was flushed with N_2 before inoculation, and the organism was grown anaerobically overnight (27°C). Then the culture was diluted into 3 liters of growth medium, and anaerobic growth was continued for 3 h. The cells were harvested by centrifugation at 14,000 × *g* for 30 min (4°C).

Spheroplasts and membrane vesicles. Freshly harvested cells were suspended in 4 liters of growth medium supplemented with 0.58 M sucrose, 15 mM $MgSO_4$, and 1.7 mM benzylpenicillin sodium salt (1,000 IU/ml) and incubated anaerobically for 3 h (27°C). During this period most cells were converted to spheroplasts, which then were harvested by centrifugation at 14,000 × *g* for 30 min (4°C). The spheroplasts were suspended to 60% (wet weight per volume) in 100

mM potassium phosphate (pH 7.5)–0.58 M sucrose–5 mM $MgSO_4$ and stored at −80°C.

The spheroplasts (60-ml) were thawed at room temperature and lysed by dilution into 1.2 liters of 50 mM potassium phosphate (pH 7.5)–10 mM EDTA–30 μg each of DNase and RNase per ml. After 5 min of incubation at 37°C, $MgSO_4$ was added to 15 mM, and the mixture was slowly stirred for 20 min. Unlysed spheroplasts were removed by centrifugation at 800 × *g* for 30 min (4°C). The vesicles were collected by centrifugation at 20,000 × *g* for 30 min (4°C) and suspended in 30 ml of vesicle buffer (100 mM potassium phosphate buffer [pH 7.5]–5 mM $MgSO_4$). The suspension was layered on top of a solution of 50% (wt/vol) sucrose in vesicle buffer in a Beckman SW27.1 rotor and centrifuged at 65,000 × *g* for 17 h (4°C). The vesicles were collected from the 0 to 50% sucrose interphase, diluted with 3 volumes of vesicle buffer, and centrifuged at 20,000 × *g* for 30 min (4°C). The vesicles were resuspended in the latter buffer to a concentration of 3.5 mg of membrane protein/ml, 18.0 mg of bovine serum albumin per ml was added, and the mixture was stored in liquid N_2. Each vesicle sample was thawed only once.

Anaerobic transport assay. The thawed vesicle suspension (above) and the transport buffer solution were bubbled with N_2 for 2 min and then flushed with N_2 (for at least 10 min) before the experiments. The transport buffer contained (per 4.5 ml): 500 μmol of potassium phosphate buffer (pH 7.5), 25 μmol of $MgSO_4$, 25 nmol of L-[^{14}C]serine (specific activity, 42.8 mCi/mmol), 50 μmol of the electron donor potassium formate, and 50 μmol of the electron acceptor TMAO. The electron donor, acceptor, or both were omitted as indicated below.

The transport buffer (4.5 ml) and N_2 gas (0.5 ml) were drawn into a disposable 5-ml syringe (Gillette, Sabre) mounted to a Stepper repetitive pipette (model 4005-100, Tridak). The anaerobic vesicle solution (0.5 ml) was drawn into a disposable 1-ml syringe, and the anaerobic transport reaction was started by injecting the vesicle solution into the 5-ml syringe through its rubber plunger. At intervals, 0.5-ml samples were membrane filtered (Sartorius; pore size, 0.45 μm). The filters were washed with 2 ml of 100 mM potassium phosphate buffer (pH 7.5)–100 mM LiCl, and radioactivity on the filters was determined by liquid scintillation counting. All steps were performed at room temperature (22°C).

Cell-free extract. Freshly harvested cells were washed twice in 50 mM potassium phosphate buffer (pH 7.0)–5 mM $MgCl_2$ in the centrifuge (14,000 × *g* for 30 min at 4°C). The washed cells, suspended to 20% (wet weight per volume) in the same buffer with 1 mM dithiothreitol, were broken by two passages through an Aminco French pressure cell (J4-3339) at 9,800 psi and then centrifuged at 39,000 × *g* for 10 min at 4°C. The supernatant was stored at −80°C. Each sample was thawed only once.

Anaerobic phosphorylation assay. The reaction mixture for the anaerobic phosphorylation assay was a modification of that of Hempfling and Hertzberg (12). The tests were performed in Thunberg tubes which were repeatedly evacuated and filled with N_2. In standard tests the main chamber contained (per 0.9 ml): 20 μmol of Tris-hydrochloride buffer (pH 7.0), 2 μmol of $MgSO_4$, 0.3 μmol of EDTA, 1 μmol of ADP, ATP-trapping system (32 μmol of glucose and 10 U of hexokinase; Sigma H 5000), carrier-free $^{32}P_i$ (90 to 180 nCi), 4 μmol of TMAO, and the electron donor (7 μmol of sodium formate or an NADH-generating system [200 μmol of ethanol, 0.2 μmol of NAD, 6 μmol of semicarbazide, and 12 U of alcohol dehydrogenase; Sigma A 7011]). The side chamber contained 0.3 ml of cell-free extract (5 to 6 mg of protein, see above). The solutions were pre-equilibrated at 25°C, and the reaction was started by mixing the contents of the chambers and stopped by the addition of 0.3 ml of 3 M perchloric acid. Organic phosphate was separated from P_i by the method of Hagihara and Lardy (9), and organic [^{32}P]phosphate formed was determined by liquid scintillation counting.

TMAO reductase assays. The NADH- or formate-dependent TMAO reductase activity (or both) of membrane vesicles and cell-free extract was determined by substituting unlabeled TMAO with same amount of [^{14}C]TMAO (specific activity, 5.0 μCi/mmol) in the transport assay and the phosphorylation assay (above) and omitting [^{14}C]serine and $^{32}P_i$, respectively. The reaction was stopped by the addition of trichloroacetic acid to 10% (wt/vol). Reduction of TMAO with reduced methyl viologen was determined in N_2-filled Thunberg tubes. The final reaction mixture contained (per 1 ml): 400 μmol of potassium phosphate buffer (pH 7.4), 0.5 μmol of methyl viologen, 10 μmol of sodium dithionite, 2 μmol of [^{14}C]TMAO, and enzyme (40 to 70 μg of membrane protein) at 22°C. The reaction was stopped by the addition of formaldehyde to 4% (wt/vol). [^{14}C]TMA formed was trapped in 0.1 M HCl by the use of the Conway and Byrne microdiffusion technique (4) and determined by liquid scintillation counting. Units of activity are micromoles of TMA produced per minute.

Metabolic inhibitors. Nigericin was a gift from Hoffmann-La Roche Inc., and the following inhibitors were purchased from Sigma Chemical Co.: carbonyl cyanide-*m*-chlorophenyl hydrazone, carbonylcyanide *p*-trifluoromethoxyphenyl hydrazone, 2-heptyl-4-hydroxyquinoline-*N*-oxide, and valinomycin; they were dissolved in ethanol, and 10 μl was added per ml of reaction mixture. Potassium cyanide and sodium azide (obtained from Merck, Sharp & Dohme) and 2,4-dinitrophenol (obtained from Sigma) were dissolved in water.

Formate dehydrogenase assay. This enzymic activity in membrane vesicles was determined spectrophotometrically at 600 nm by phenazine methosulfate-mediated reduction of dichlorophenolindophenol as described previously (21). Units of specific activity are micromoles of dichlorophenolindophenol reduced per milligram of protein per minute at 22°C.

Other methods. Protein was determined by the method of Lowry et al. (22) with bovine serum albumin as the standard. Amino acid chromatography was performed on a Jeol JLC-6AH amino acid analyzer. Liquid scintillation counting was performed in Hydro Luma (Lumac Systems A.G.) with a Packard Tri-Carb 2425 liquid scintillation spectrometer.

Radioactive chemicals. L-[^{14}C]serine (specific activity, 170 mCi/mmol) and [^{14}C]TMA hydrochloride (specific activity, 1.8 mCi/mmol) were purchased from New England Nuclear Corp. The [^{14}C]TMA reagent was purified by the Conway and Byrne microdiffusion technique (4) as previously described (30) and oxidized

to [^{14}C]TMAO with H_2O_2 (6). Carrier-free $^{32}P_i$ was obtained from the Institutt for energiteknikk, Kjeller, Norway.

RESULTS

TMAO-dependent L-serine transport. Electron transfer-dependent transport systems of anaerobically grown cells are often more labile than those of aerobically grown cells; the systems are easily inactivated during osmotic lysis of spheroplasts and washing of membrane vesicles (17, 19). This problem was overcome by using vesicles derived from penicillin-produced spheroplasts by the procedures described above.

The purified membrane vesicles were grayish white. In the light microscope they appeared as transparent "ghosts" with a diameter of approximately 0.6 to 1.2 μm, whereas the spheroplast diameter was approximately 1.2 to 1.8 μm. This indicated that the cytoplasmic membrane of the purified vesicles had not been fragmented to any large extent during the osmotic lysis.

The time course of anaerobic serine uptake in the membrane vesicles in presence of TMAO and formate is shown in Fig. 1. The accumulation of serine reached its peak value after approximately 1 min, whereas the reduction of TMAO to TMA was linear with time for at least 20 min (Fig. 2). In the absence of both TMAO and formate the serine uptake was greatly diminished; the uptake was only slightly stimulated by the addition of either the electron donor or acceptor alone. Membrane vesicles without bovine serum albumin did not accumulate serine (Fig. 1). Bovine serum albumin is reported to remove from membranes free fatty acids which otherwise can act as proton conductors (20, 33).

Serine was not chemically modified by the membrane vesicles. All ^{14}C-labeled material accumulated by the vesicles could be extracted with 1% (wt/vol) picric acid and had the same retention time as standard serine when chromatographed by use of an amino acid analyzer.

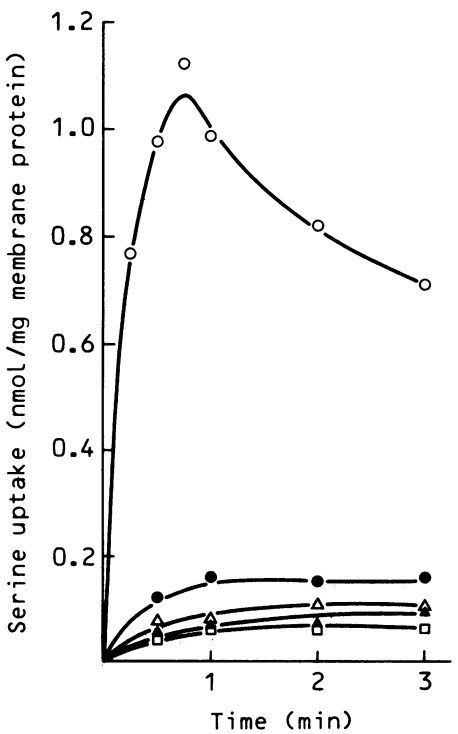

FIG. 1. Anaerobic serine uptake in membrane vesicles from *Proteus* sp. strain NTHC153 grown anaerobically with glucose and TMAO. Vesicles treated with bovine serum albumin and (○) formate and TMAO, (●) formate, (△) TMAO, or (▲) no additions. Vesicles without bovine serum albumin and (□) formate and TMAO.

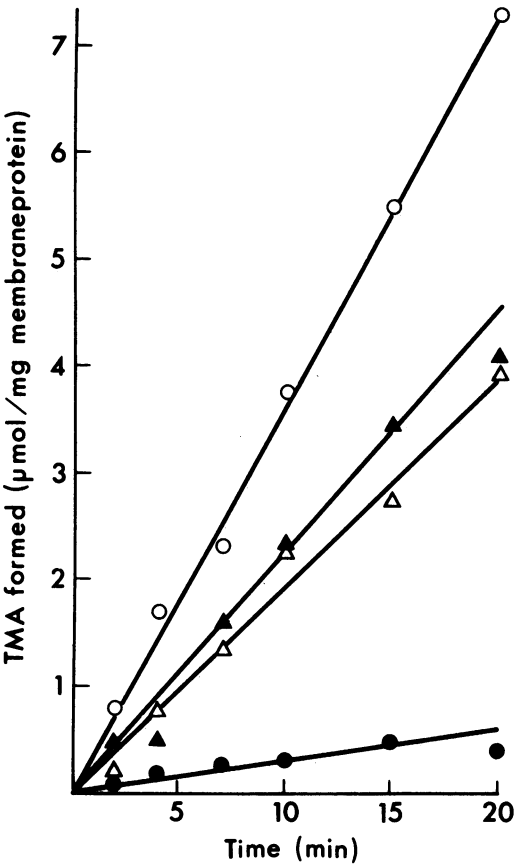

FIG. 2. Effect of electron transfer chain inhibitor and chelating agents on the reduction of TMAO with formate by transport-active membrane vesicles. Symbols: ○, control with no addition; ▲, 2 mM potassium cyanide: △, 10 mM sodium azide; and ● 80 μM 2-heptyl-4-hydroxyquinoline-*N*-oxide.

To investigate the nature of the energy transduction between the formate-TMAO redox reaction and the serine uptake, the effects of various metabolic inhibitors were tested. The serine uptake was inhibited by the protonophores (uncouplers) carbonyl cyanide-*m*-chlorophenyl hydrazone (5 μM) and carbonylcyanide *p*-trifluoromethoxyphenyl hydrazone (4 μM) and by the ionophores nigericin (1 μM) and valinomycin (2 μM); at the concentrations tested carbonyl cyanide-*m*-chlorophenyl hydrazone and valinomycin were the most effective inhibitors (Fig. 3). At these concentrations none of the inhibitors had any effect on the formate-dependent TMAO reduction.

The electron transfer chain inhibitor 2-heptyl-4-hydroxyquinoline-*N*-oxide (80 μM) and the chelating agents potassium cyanide (2 mM) and sodium azide (10 mM) inhibited formate-dependent TMAO reduction (Fig. 2); they also inhibited serine transport.

Evidence of only cytochrome *b* was found in the vesicles. This was obtained by extraction with HCl-acetone, with measurement of the alkaline pyridine hemochrome difference spectra (28, 29). The acid acetone-soluble fraction revealed three distinct peaks with maxima at 557, 522, and 420 nm, corresponding closely to the α, β, and γ bands, respectively, of protoheme of cytochromes *b* (15). No evidence of cytochrome *c* was found in the acid acetone insoluble fraction of *Proteus* sp. vesicles.

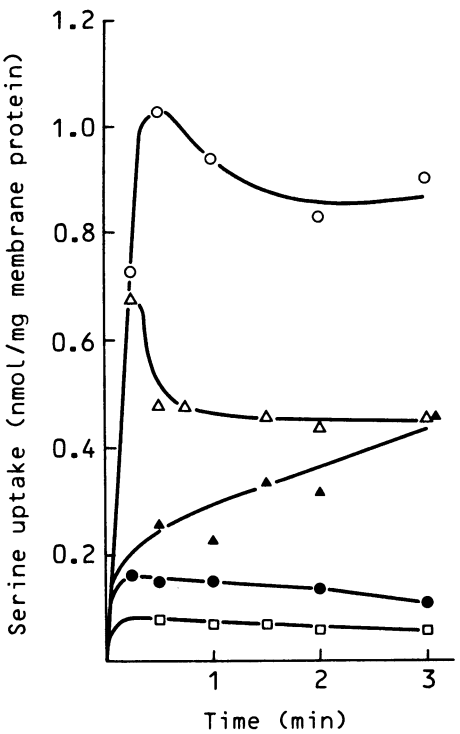

FIG. 3. Effect of protonophores and ionophores on anaerobic serine transport in membrane vesicles energized by electron transfer from formate to TMAO. Symbols: ○, control with no addition; □, 5 μM carbonyl cyanide-*m*-chlorophenyl hydrazone; ▲, 4 μM carbonylcyanide *p*-trifluoromethoxyphenyl hydrazone; △, 1 μM nigericin, and ●, 2 μM valinomycin.

TABLE 1. Formate dehydrogenase and TMAO reductase activity of membrane vesicles assayed with artificial electron acceptor and donor, respectively[a]

Enzyme	Electron donor	Electron acceptor	Sp act (μmol/mg of protein/min)
Formate dehydrogenase		PMS	1.5
TMAO reductase	MV		0.6

[a] PMS, Phenazine methosulfate; MV, reduced methyl viologen.

The formate dehydrogenase complex of *E. coli* that takes part in the electron transfer from formate to nitrate reduces redox dyes such as phenazine methosulfate, which accepts two electrons (18, 21). The present vesicles from cells grown anaerobically with TMAO had high activity of a similar formate dehydrogenase complex (Table 1). TMAO reductase of the *Proteus* sp. vesicles accepted electrons from reduced methyl viologen (Table 1), as does the TMAO reductase of *E. coli* (27).

TMAO-dependent phosphorylation. We assayed for ATP synthesis coupled with electron transfer to TMAO in cellfree extract prepared in a French pressure cell. Figure 4A and B shows the time courses of TMAO disappearance and formation of organic [^{32}P]phosphate in the anaerobic reaction mixtures with NADH-generating system and formate as electron donors, respectively. The TMAO-dependent organic [^{32}P]phosphate shown is the amount of labeled organic phosphate formed in the complete reaction mixture minus the background phosphorylation in reaction mixture without TMAO. It should be noted that the formation of TMAO-dependent organic phosphate ceased when the TMAO reduction was complete, and that it was consistently higher with NADH than with formate as electron donor. The background phosphorylation continued throughout the whole observation period and was the same with both electron donors (and without any electron donor).

A typical example of the P/2e$^-$ ratios obtained in cell-free extracts with a good coupling is listed in Table 2; the P/2e$^-$ ratios with NADH and formate as electron donors were 0.3 and 0.1, respectively. The data in Table 2 also show that TMAO stimulated phosphorylation very little in the absence of added electron donor, and that little organic [^{32}P]phosphate accumulated when

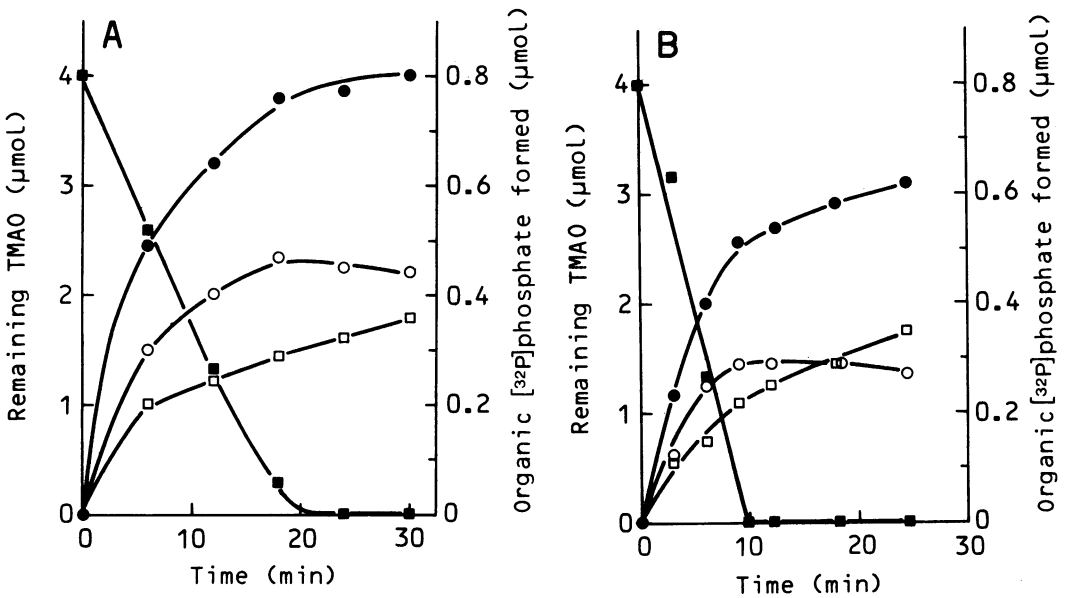

FIG. 4. Anaerobic synthesis of organic [^{32}P]phosphate and reduction of TMAO by cell-free extract (6 mg of protein) of *Proteus* sp. strain NTHC153 cells disrupted in French pressure cell. Electron donors for the reduction of TMAO were (A) NADH-generating system and (B) formate. The reaction mixtures contained $^{32}P_i$, ADP, ATP-trapping system. Symbols: ●, organic [^{32}P]phosphate in full reaction mixture with electron donor and TMAO; □, organic [^{32}P]phosphate in control without TMAO; ○, TMAO-dependent organic [^{32}P]phosphate (see text); and ■, TMAO remaining in reaction mixture.

ADP and the ATP-trapping system were omitted from the reaction mixture. The extracts contained hydrolytic ATPase activity (data not shown).

2-Heptyl-4-hydroxyquinoline-*N*-oxide inhibited both the NADH-dependent TMAO reduction and TMAO-dependent phosphorylation. The uncouplers 2,4-dinitrophenol, carbonyl cyanide-*m*-chlorophenyl hydrazone, and carbonylcyanide *p*-trifluoromethoxyphenyl hydrazone inhibited the latter only (Table 3). None of these inhibitors affected the background phosphorylation.

Although the results are not shown, it is noteworthy that all NADH- and formate-dependent TMAO reductase activity was found in the membraneous fraction after high-speed centrifugation of the cell-free extract. The membranes did not, however, catalyze phosphorylation, indicating the need for coupling factors for ATP synthesis (25). Neither did transport-active *Proteus* sp. vesicles display any phosphorylation activity.

DISCUSSION

The present study shows that TMAO reduction in *Proteus* sp. strain NTHC153 fulfills the essential features of anaerobic respiration in bacteria. Several independent observations lead

TABLE 2. Determination of phosphorylation efficiencies, P/2e$^-$ ratios, of anaerobic TMAO respiration in cell-free extract (prepared in French pressure cell) of *Proteus* sp. strain NTHC153 cells grown anaerobically with glucose and TMAO[a]

Electron donor	TMAO added	ADP and ATP-trapping system added	Organic [^{32}P]-phosphate synthesized (μmol)	TMAO reduced (μmol)	Net P/2e$^-$ ratio
None	−	+	0.18		
None	+	+	0.23	0.1	
NADH	−	+	0.23		
NADH	+	+	0.68	1.7	0.3
NADH	−	−	0.04		
NADH	+	−	0.11	1.5	
Formate	−	+	0.22		
Formate	+	+	0.44	3.0	0.1

[a] The reaction mixture contained 5.2 mg of cell protein, and the incubation time was 10 min.

TABLE 3. Effect of inhibitors on the NADH- and TMAO-dependent phosphorylation and the NADH-dependent TMAO reduction in cell-free extract of *Proteus* sp. strain NTHC153 cells disrupted in French pressure cell

Inhibitor[a]	Inhibitor concn (μM)	nmol of inhibitor/mg of protein	% Inhibition	
			TMAO- and NADH-dependent phosphorylation	NADH-dependent TMAO reduction
HOQNO	2.5	0.6	20	60
	5.0	1.2	30	70
	12.5	3.0	70	80
Dinitrophenol	50	12	50	0
	100	24	60	0
CCCP	100	24	30	0
FCCP	100	24	30	0

[a] HOQNO, 2-Heptyl-4-hydroxyquinoline-*N*-oxide; CCCP, carbonyl cyanide-*m*-chlorophenyl hydrazone; FCCP, carbonylcyanide *p*-trifluoromethoxyphenyl hydrazone.

to this conclusion, which is consistent with the previously published growth data for the organism (32).

An energization of the cytoplasmic membrane by electron transfer from formate to TMAO is evident from the observed serine transport against the concentration gradient of serine in membrane vesicles. Assuming a vesicle volume of 2.2 μl per mg of membrane protein (14), the ratio of the peak concentration of serine inside the vesicles versus that initially outside (5 μM) is approximately 100 (Fig. 1 and 3). As expected for active amino acid transport in bacterial cells the serine transport is sensitive to metabolic inhibitors destroying the transmembrane proton gradient and electrical potential. It is previously shown for *E. coli* that the electrochemical proton gradient is involved in driving active transport under anaerobic conditions in a similar way as under aerobic conditions (2).

Anaerobic electron transfer from formate and NADH to TMAO is coupled with ATP synthesis in cell-free extracts (prepared in a French pressure cell) of *Proteus* sp. strain NTHC153. This ATP synthesis is sensitive to uncouplers of oxidative phosphorylation. The P/2e$^-$ ratio of 0.3 determined for the reduction of TMAO with NADH is close to the values determined for the reduction of nitrate and oxygen with NADH in subcellular preparations of the related bacterium *E. coli*; i.e., about 0.3 (26) and 0.6 (12), respectively. The observed efficiencies of oxidative phosphorylation by subcellular preparations of bacteria are generally much lower than that of intact mitochondria oxidizing identical substrates. The main reason for this seems to be that an in vitro assay of oxidative phosphorylation depends on the presence of everted membrane vesicles because of the impermeability of bacterial membranes to nucleotide cofactors (10). The transport-active *Proteus* sp. vesicles do not catalyze oxidative phosphorylation.

In intact cells of *E. coli* the phosphorylation efficiency is reported to be similar to that of mitochondria (12), and the growth data for *Proteus* sp. strain NTHC153 indicate that the P/2e$^-$ ratio for the formate-TMAO redox reaction is at least 1 (32). Thus, the present in vitro data do not give the true values for the phosphorylation efficiencies in TMAO respiration.

The electron transfer chain to TMAO in *Proteus* sp. strain NTHC153 seems to be simpler than the corresponding electron transfer chain in *E. coli* which contains both *b*- and *c*-type cytochromes (13, 28), but this does not appear to result in a lower in vivo phosphorylation efficiency since the anaerobic molar growth yield on formate with TMAO as terminal electron acceptor is 10.3 for *Proteus* sp. strain NTHC153 (32) and 7.0 for *E. coli* (34). Because of its simplicity, the TMAO reductase system in *Proteus* sp. strain NTHC153 may be useful for further studies of the chemiosmotic mechanism.

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LITERATURE CITED

1. **Boonstra, J., M. T. Huttunen, W. N. Konings, and H. R. Kaback.** 1975. Anaerobic transport in *Escherichia coli* membrane vesicles. J. Biol. Chem. **250**:6792–6798.
2. **Boonstra, J., and W. N. Konings.** 1977. Generation of an electrochemical proton gradient by nitrate respiration in membrane vesicles from anaerobically grown *Escherichia coli*. Eur. J. Biochem. **78**:361–368.
3. **Boyer, P. D., B. Chance, L. Ernster, P. Mitchell, E. Racker, and E. C. Slater.** 1977. Oxidative phosphorylation and photophosphorylation. Annu. Rev. Biochem. **46**:955–1026.
4. **Conway, E. J., and A. Byrne.** 1933. An absorption apparatus for the micro-determination of certain volatile substances. I. The micro-determination of ammonia. Biochem. J. **27**:419–429.
5. **Cox, J. C., M. T. Madigan, J. L. Favinger, and H. Gest.** 1980. Redox mechanism in "oxidant-dependent" hexose fermentation by *Rhodopseudomonas capsulata*. Arch. Biochem. Biophys. **204**:10–17.

6. **Dunstan, W. R., and E. Goulding.** 1899. The action of hydrogen peroxide on secondary and tertiary aliphatic amines. Formation of hydroxylamines and oxamines. J. Chem. Soc. **75**:1004–1011.
7. **Fillingame, R. H.** 1980. The proton-translocating pumps of oxidative phosphorylation. Annu. Rev. Biochem. **49**:1079–1113.
8. **Groninger, H. S.** 1959. The occurrence and significance of trimethylamine oxide in marine animals. U.S. Fish Wildl. Serv. Spec. Sci. Rep. Fish. **333**:1–22.
9. **Hagihara, B., and H. A. Lardy.** 1960. A method for the separation of orthophosphate from other phosphate compounds. J. Biol. Chem. **235**:889–894.
10. **Harold, F. M.** 1972. Conservation and transformation of energy by bacterial membranes. Bacteriol. Rev. **36**:172–230.
11. **Harold, F. M.** 1978. Vectorial metabolism, p. 463–521. *In* L. N. Ornston and J. R. Sokatch (ed.), The bacteria, vol. 6. Academic Press, Inc., New York.
12. **Hempfling, W. P., and E. L. Hertzberg.** 1979. Techniques for measurement of oxidative phosphorylation in intact bacteria and in membrane preparations of *Escherichia coli*. Methods Enzymol. **55**:164–175.
13. **Ishimoto, M., and O. Shimokawa.** 1978. Reduction of trimethylamine N-oxide by *Escherichia coli* as anaerobic respiration. Z. Allg. Mikrobiol. **18**:173–181.
14. **Kaback, H. R., and E. M. Barnes.** 1971. Mechanisms of active transport in isolated membrane vesicles. II. The mechanism of energy coupling between D-lactic dehydrogenase and β-galactoside transport in membrane preparations from *Escherichia coli*. J. Biol. Chem. **246**:5523–5531.
15. **Kamen, M. D., and T. Horio.** 1970. Bacterial cytochromes: I. Structural aspects. Annu. Rev. Biochem. **39**:673–700.
16. **Kim, K. E., and G. W. Chang.** 1974. Trimethylamine oxide reduction by *Salmonella*. Can. J. Microbiol. **20**:1745–1748.
17. **Konings, W. N.** 1979. Energization of solute transport in membrane vesicles from anaerobically grown bacteria. Methods Enzymol. **56**(Part C):378–388.
18. **Konings, W. N., and J. Boonstra.** 1977. Anaerobic electron transfer and active transport in bacteria. Curr. Top. Membr. Transp. **9**:177–231.
19. **Konings, W. N., and H. R. Kaback.** 1973. Anaerobic transport in *Escherichia coli* membrane vesicles. Proc. Natl. Acad. Sci. U.S.A. **70**:3376–3381.
20. **Lai, J. S., S. I. Okuda, and H. Takahashi.** 1978. Stimulative effect of bovine serum albumin on the transport activity of serine in membrane vesicles from *Escherichia coli*. J. Gen. Appl. Microbiol. **24**:251–254.
21. **Lester, R. L., and J. A. DeMoss.** 1971. Effects of molybdate and selenite on formate and nitrate metabolism in *Escherichia coli*. J. Bacteriol. **105**:1006–1014.
22. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
23. **Miki, K., and E. C. C. Lin.** 1975. Anaerobic energy-yielding reaction associated with transhydrogenation from glycerol 3-phosphate to fumarate by an *Escherichia coli* system. J. Bacteriol. **124**:1282–1287.
24. **Miki, K., and E. C. C. Lin.** 1975. Electron transport chain from glycerol-3-phosphate to nitrate in *Escherichia coli*. J. Bacteriol. **124**:1288–1294.
25. **Ota, A.** 1965. Oxidative phosphorylation coupled with nitrate respiration. III. Coupling factors and mechanism of oxidative phosphorylation. J. Biochem. (Tokyo) **58**: 137–144.
26. **Ota, A., T. Yamanaka, and K. Okunuki.** 1964. Oxidative phosphorylation coupled with nitrate respiration. II. Phosphorylation coupled with anaerobic nitrate reduction in a cell-free extract of *Escherichia coli*. J. Biochem. (Tokyo) **55**:131–135.
27. **Sakaguchi, M., and A. Kawai.** 1977. Electron donors and carriers for the reduction of trimethylamine N-oxide in *Escherichia coli*. Bull. Jpn. Soc. Sci. Fish. **43**:437–442.
28. **Sakaguchi, M., and A. Kawai.** 1978. Presence of b- and c-type cytochromes in membrane of *Escherichia coli* induced by trimethylamine N-oxide. Bull. Jpn. Soc. Sci. Fish. **44**:999–1002.
29. **Sperry, J. F., and T. D. Wilkins.** 1976. Cytochrome spectrum of an obligate anaerobe, *Eubacterium lentum*. J. Bacteriol. **125**:905–909.
30. **Strøm, A. R.** 1979. Biosynthesis of trimethylamine oxide in calanoid copepods. Seasonal changes in trimethylamine monooxygenase activity. Mar. Biol. **51**:33–40.
31. **Strøm, A. R., and H. Larsen.** 1979. Anaerobic fish spoilage by bacteria. I. Biochemical changes in herring extracts. J. Appl. Bacteriol. **46**:531–543.
32. **Strøm, A. R., J. A. Olafsen, and H. Larsen.** 1979. Trimethylamine oxide: a terminal electron acceptor in anaerobic respiration of bacteria. J. Gen. Microbiol. **112**:315–320.
33. **Wojtczak, L., and A. L. Lehninger.** 1961. Formation and disappearance of an endogenous uncoupling factor during swelling and contraction of mitochondria. Biochim. Biophys. Acta **51**:442–456.
34. **Yamamoto, I., and M. Ishimoto.** 1977. Anaerobic growth of *Escherichia coli* on formate by reduction of nitrate, fumarate, and trimethylamine N-oxide. Z. Allg. Mikrobiol. **17**:235–242.