Short Communication

REDUCTION OF *N*-OXIDE AND *S*-OXIDE COMPOUNDS BY *ESCHERICHIA COLI*

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The anaerobic growth of *Escherichia coli* is supported by energy production coupled to the reduction of electron acceptors including nitrate, fumarate, trimethylamine *N*-oxide (TMAO), and dimethyl sulfoxide (DMSO) (1, 9, 11, 18). Reduction of tetrahydrothiophene oxide (13) also effectively elevates the growth levels of the cell in anaerobiosis. So far, terminal enzymes in electron transport, nitrate reductase (4, 8), fumarate reductase (5), TMAO reductase (23), and DMSO reductase (19) have been purified and characterized as distinct enzymes. Among these enzymes TMAO reductase and DMSO reductase have broad specificity for substrates; TMAO reductase can reduce TMAO, adenosine *N*-oxide, γ -picoline *N*-oxide, hydroxylamine and chlorate (23), and these compounds, except adenosine *N*-oxide, and *S*-oxides including DMSO and L-methionine sulfoxide are utilized by DMSO reductase as substrates (19). In this study we examined the effects of some *N*-oxide or *S*-oxide compounds on the anaerobic growth of *E. coli*, and investigated whether enzymes specific for the reduction of the respective oxides would be induced.

E. coli strain K10 was used in this study. The cells were transferred from a slant culture to LB medium (14) and incubated at 37°C overnight. The culture obtained was inoculated into a fresh LB in a flask capped with a rubber septum with glass tubings. The size of inoculation was 2.5%. The concentration of each oxide compound was 30 mM in LB medium. The cells were anaerobically grown by bubbling nitrogen and harvested by centrifugation at $10,000 \times g$ for 10 min. The optical density at 660 nm (OD_{660}) of the cultures was measured in a 10×10 mm cuvette with a spectrophotometer (Jasco Uvidec 340). When the OD_{660} was higher than 0.5, the culture was diluted with saline for the measurement. The amount of cells grown was estimated on the basis that 1 OD_{660} corresponds to 0.40 mg of dry

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cells per ml (20).

The cells suspended in 50 mM Tris-HCl, pH 7.4, were sonicated at 20 kHz, 93 W for 2.5 min with a sonicator (Tomy UR-150P) and centrifuged at $10,000 \times g$ for 15 min. The supernatant obtaining (S_{10}) was further centrifuged at $100,000 \times g$ for 90 min. The supernatant was used as S_{100} . The precipitate was washed with the buffer then solubilized with 1% sodium lauroyl sarcosinate. After ultracentrifugation the supernatant was used as P_{100} .

The reductase activities were determined by measuring the decrease of the absorbance at 315 nm of dithionite at 30°C with a spectrophotometer (Jasco Uvidex 340), as described previously (17). The reaction mixtures for assaying reductase activities of TMAO, γ -picoline *N*-oxide and adenosine *N*-oxide contained (0.6 ml): 50 mM sodium acetate, pH 5.5, 2 mM benzyl viologen, 3.3 mM sodium dithionite, 5 mM potassium bicarbonate, and the respective oxides. Either 15 mM TMAO, 5 mM γ -picoline *N*-oxide, or 5 mM adenosine *N*-oxide was used as the substrate. To assay reductase activities with 2 mM DMSO and 5 mM L-methionine sulfoxide, 100 mM potassium phosphate, pH 6.8, and 0.2 mM benzyl viologen were substituted for the acetate buffer and 2 mM benzyl viologen. Protein was determined by the method of Lowry et al. (12) with bovine serum albumin as the standard.

To visualize enzymes, cell-free extracts were subjected to polyacrylamide disc gel electrophoresis (6) without detergents, then the gels were stained for enzyme activities (17). The reaction mixture (50 ml) for enzyme activity staining was composed of 50 mM Tris-HCl, pH 7.4, 1 mM methyl viologen, 0.4 g of sodium dithionite, 0.1 g of potassium bicarbonate, and a substrate. Either 15 mM TMAO, 10 mM γ -picoline *N*-oxide, 10 mM adenosine *N*-oxide, 5 mM DMSO, or 10 mM L-methionine sulfoxide was used as the substrate. To stain DMSO and methionine sulfoxide reductases, 100 mM Tris-maleate, pH 6.9, was used instead of Tris-HCl,

Fig. 1. Anaerobic growth of *E. coli* in LB medium with or without oxidized compounds. Cultures were incubated at 37°C with nitrogen-bubbling. ■, cell growth without oxidized compounds; △, with 30 mM γ -picoline *N*-oxide; ○, with 30 mM adenosine *N*-oxide; ×, with 30 mM L-methionine sulfoxide; □, with 30 mM TMAO; ▲, with 30 mM DMSO.

Table 1. Reductase activities with TMAO, DMSO, γ -picoline *N*-oxide, adenosine *N*-oxide, and L-methionine sulfoxide in cell-free extracts from cells grown anaerobically with some oxide compounds.

Cells grown with	Fraction	Protein (mg)	Reductase activities											
			TMAO		DMSO		γ -Picoline <i>N</i> -oxide		Adenosine <i>N</i> -oxide		L-Methionine sulfoxide			
			Specific ^a	Total ^b	Specific	Total	Specific	Total	Specific	Total	Specific	Total		
None	S ₁₀	69.6	344	100	59.4	100	146	100	57.5	100	41.7	100		
	S ₁₀₀	47.5	128	25.4	23.4	26.9	51.1	23.9	40.1	47.6	27.0	44.4		
	P ₁₀₀	10.6	1,440	63.7	142	36.3	566	59.1	292	77.2	151	55.4		
γ -Picoline <i>N</i> -oxide	S ₁₀	126	493	100	67.3	100	438	100	ND ^c	—	ND	—		
	S ₁₀₀	74.1	381	45.5	43.3	38.0	202	27.2	ND	—	ND	—		
	P ₁₀₀	14.2	1,870	42.8	196	32.9	2,150	55.5	ND	—	ND	—		
Adenosine <i>N</i> -oxide	S ₁₀	122	454	100	71.4	100	ND	—	66.4	100	ND	—		
	S ₁₀₀	77.3	120	16.8	28.9	25.6	ND	—	59.6	56.9	ND	—		
	P ₁₀₀	16.6	1,560	46.6	190	36.2	ND	—	283	57.8	ND	—		
L-Methionine sulfoxide	S ₁₀	40.5	450	100	71.7	100	ND	—	ND	—	65.6	100		
	S ₁₀₀	24.8	161	21.9	26.6	22.7	ND	—	ND	—	53.2	49.7		
	P ₁₀₀	7.2	1,290	50.8	146	36.2	ND	—	ND	—	177	48.0		

^a Specific activity, nmol/min/mg of protein.

^b Total activity. The activity in S₁₀ was represented as 100%.

^c Not determined.

pH 7.4. To stop the reductase activities, the gels were soaked in 1% triphenyltetrazolium chloride; enzymes were visualized as colorless bands on a red background. Values of relative mobility (R_m) were obtained with respect to bromophenol blue. The S_{100} from TMAO-grown cells contained three bands which showed TMAO reductase activity with an R_m of 0.24 (TMAO reductase I), 0.36 (TMAO reductase III), and 0.50 (DMSO reductase), as observed previously (17). These values of R_m were not affected by adding *N*-lauroyl sarcosinate to S_{100} . The bands with $R_m=0.24$ and 0.50 were found in the P_{100} fraction which contained the detergent.

E. coli was grown anaerobically on LB broth in the absence or presence of γ -picoline *N*-oxide, adenosine *N*-oxide, L-methionine sulfoxide, TMAO, and DMSO at 30 mM (Fig. 1). All oxides elevated the levels of cell growth but the doubling time of the growth was not greatly affected: the amounts of cells grown were 0.18 mg of dry cells/ml in the culture without oxides, 0.29 mg/ml with TMAO, 1.1 mg/ml with adenosine *N*-oxide, 0.25 mg/ml with γ -picoline *N*-oxide, 0.28 mg/ml with DMSO, and 0.27 mg/ml with L-methionine sulfoxide. The doubling times of the cell growth in all cultures were 32–38 min during 0.5–2 h of incubation. Almost the same levels of the cell growth in the cultures with oxide compounds, except adenosine *N*-oxide, suggest that the growth yields of the respective oxides may be equal. TMAO reduction by formate (21) or by hydrogen (22) has been shown to be coupled with energy production; 1 mol of ATP is presumably produced in the reduction of 1 mol of TMAO. Adenosine *N*-oxide caused the highest growth, 4-fold that in the TMAO culture. This may be due to further metabolism of adenosine *N*-oxide. Adenosine and adenine have been identified as the products in the reduction of adenosine *N*-oxide by a soluble enzyme isolated from *E. coli* (16). An increase in the growth of *E. coli* was observed in the presence of either adenosine or D-ribose

Fig. 2. Reductase activity staining on polyacrylamide gel. The S_{100} and P_{100} fractions prepared from cells grown anaerobically with oxide compounds were electrophoresed on polyacrylamide disc gels, then the gels were stained for reductase activities with the oxide used for the cell growth (details were described in the text). 1 and 2, cells grown anaerobically without oxide compounds and stained for TMAO reductase activity; 3 and 4, cells grown and activity stained by γ -picoline *N*-oxide reduction; 5 and 6, cells grown and activity stained by adenosine *N*-oxide reduction; 7 and 8, cells grown and activity stained by L-methionine sulfoxide reduction. Odd numbers of gels indicate S_{100} fractions and even numbers P_{100} fractions. Arrows indicate positions of bromophenol blue as a running marker.

under similar growth conditions; the amounts of cells grown were 0.51 mg/ml with adenosine and 0.25 mg/ml with D-ribose.

The activities of γ -picoline *N*-oxide reductase, adenosine *N*-oxide reductase, L-methionine sulfoxide reductase, TMAO reductase, and DMSO reductase were demonstrated in cell-free extracts prepared from the cells grown in LB medium without oxide compounds (Table 1). When polyacrylamide disc gel electrophoresis was carried out then the gels were stained for enzyme activities, reductase activity with TMAO was found at the *Rm* position of 0.49 or 0.50 in P₁₀₀ (Fig. 2, lane 2). Reductase activities with DMSO, γ -picoline *N*-oxide, or L-methionine sulfoxide were also stained at the same position (data not shown), indicating that in the cells grown anaerobically without oxide compounds these reductase activities including TMAO-reducing activity are attributed to the membrane-bound DMSO reductase.

In S₁₀ from γ -picoline *N*-oxide-grown cells, the reductase activity with γ -picoline *N*-oxide was 3-fold higher than that in the cells grown without oxide compounds (Table 1). Either reductase activity with TMAO or that with DMSO was observed at an equal level in both cells. In S₁₀₀, three bands were faintly stained with γ -picoline *N*-oxide as a substrate during incubation for 20 min; values of *Rm* were 0.27, 0.32, and 0.37 (Fig. 2, lane 3). In P₁₀₀, two bands with *Rm*=0.23 and 0.50 were found (Fig. 2, lane 4). These bands were also stained by TMAO reductase activity (data not shown). These findings may suggest that expression of the structural gene *torA* for TMAO reductases (15) is induced by γ -picoline *N*-oxide to a small extent.

Neither the specific activity of adenosine *N*-oxide reductase nor that of L-methionine sulfoxide reductase was affected by adding each oxide compound to the anaerobic cultures (Table 1). A single band with *Rm*=0.50 was stained by the reductase activity of L-methionine sulfoxide in P₁₀₀ prepared from the L-methionine sulfoxide-grown cells (Fig. 2, lane 8). But no band stained by adenosine *N*-oxide reduction was found in cells grown anaerobically with adenosine *N*-oxide (Fig. 2, lane 5, 6).

TMAO reductase was induced in the culture with TMAO as reported (17) with concomitant increases of activities of γ -picoline *N*-oxide reductase and adenosine *N*-oxide reductase (data not shown). In cells grown with DMSO the sulfoxide reductase activity was demonstrated at a level equal to that in cells grown without oxide compounds (data not shown).

In this study, the anaerobic growth of *E. coli* was stimulated by the presence of γ -picoline *N*-oxide, adenosine *N*-oxide, and L-methionine sulfoxide, in addition to the positive effect on the cell growth of TMAO (10) and DMSO (3). Since the activities of γ -picoline *N*-oxide reductase, adenosine *N*-oxide reductase, L-methionine sulfoxide reductase, TMAO reductase, and DMSO reductase occurred in cells grown with respective compounds, the cell growth seems to be supported by energy production coupled to the reduction of those compounds. However, there was induction of neither adenosine *N*-oxide reductase nor of L-methionine sulfoxide reductase, as of DMSO reductase. In γ -picoline *N*-oxide-grown cells, both DMSO reductase and TMAO reductase are likely to serve for the reduction of γ -picoline

N-oxide. The activity of adenosine *N*-oxide reductase was not detected clearly by the staining technique, though the enzyme reducing adenosine *N*-oxide has been purified from a soluble fraction of *E. coli* grown anaerobically without any oxide compounds (16). DMSO reductase purified from the *E. coli* membrane does not catalyze the reduction of adenosine *N*-oxide (19). Reduction of L-methionine sulfoxide was catalyzed by the constitutive membrane-bound enzyme DMSO reductase (Fig. 2, lane 8). On the other hand, L-methionine sulfoxide reductase has been purified from S₁₀₀ of the *E. coli* strain B (7). This enzyme is molecularly distinct from either DMSO reductase (19) or TMAO reductase (23). Since both L-methionine sulfoxide reductase and DMSO reductase are constitutive enzymes, each enzyme seems to serve in reduction of L-methionine sulfoxide added to a growth medium. It is an issue whether each enzyme would be involved in energy conservation to increase the growth of cells. DMSO reductase plays a role in proton translocation (2), while participation of L-methionine sulfoxide reductase in energy production remains to be studied.

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