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Effects of Selenium Compounds on Formate Metabolism and Coincidence of Selenium-75 Incorporation and Formic Dehydrogenase Activity in Cell-Free Preparations of *Escherchia coli*

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The effects of selenite, selenocystine, and selenomethionine in a defined growth medium on formic dehydrogenase biosynthesis in aerobically and anaerobically grown *Escherichia coli* have been studied. Sucrose gradient centrifugation of a partially purified enzyme demonstrated a coincidence of ^{75}Se incorporation and formic dehydrogenase activity.

The requirement for selenium in trace amounts in a defined growth medium for the biosynthesis of formic dehydrogenase by aerobically grown *Escherichia coli* has been reported (1, 7). Recently, Lester and DeMoss (4) demonstrated that anaerobic cultures also need selenite in the growth medium to have enzyme activity. We have not only confirmed the reports concerning selenite, but have also found that selenocystine and selenomethionine can substitute for selenite in the growth medium for both aerobic and anaerobic cultures.

Four different strains of *E. coli* [Crookes (ATCC 8739), B (ATCC 11303), University of Washington 325b, and University of Illinois K-12, 58-161] were examined for formic dehydrogenase activity after they were cultured aerobically on a reciprocating platform shaker at 30 C or anaerobically in stationary culture at 37 C in a defined medium containing: glucose, 1.0 g; K_2HPO_4, 1.6 g; KH_2PO_4, 1.6 g; $(NH_4)_2SO_4$, 0.4 g; NaCl, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.05 g; $FeSO_4 \cdot 7H_2O$, 5.0 μg; $Na_2MoO_4 \cdot 2H_2O$, 1.0 μg; and 100 ml of glass-distilled water. The glucose was sterilized separately from the salt solution and added to the medium just prior to inoculation. When required, each 100 ml of the defined medium was supplemented with 1.0 μg of sodium selenite, 3.8 μg of seleno-DL-methionine, or 3.4 μg of seleno-DL-cystine. Cells were harvested by centrifugation and washed twice with 0.05 M potassium phosphate buffer, pH 6.8. Formic dehydrogenase activity was determined manometrically in a Warburg respirometer at 30 C by using either methylene blue or benzyl viologen as electron acceptor and measuring the rate of carbon dioxide evolution from formate under an atmosphere of nitrogen.

The presence of selenium in the growth medium is an essential requirement for the biosynthesis of formic dehydrogenase by *E. coli* (Table 1). No attempt was made to remove contaminating selenium from the reagent-grade chemicals used in preparing the defined growth medium since the amount of selenium present in those chemicals did not stimulate significant enzyme activity. This agrees with the report by Lester and DeMoss (4) although their defined medium had a different composition.

All four strains of *E. coli* grown in stationary culture exhibited enzyme activity with methylene blue or benzyl viologen as electron acceptor only when selenite, selenocystine, or selenomethionine was added to the defined growth medium. However, enzyme activity in cells from the selenomethionine medium was only half that of cells from selenite or selenocystine medium. When the growth medium was deprived of selenium, the cultures did not exhibit detectable enzyme activity.

Cells grown aerobically in the medium supplemented with selenite or selenocystine had good enzyme activity using methylene blue as

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TABLE 1. *Effect of selenium compounds on formic dehydrogenase activity in Escherichia coli*[a]

Escherichia coli strain	Growth condition	Electron acceptor	Specific activity (μliters of CO_2 evolved per mg dry cells per hr)			
			Selenite	Selenocystine	Selenomethionine	No selenium
Strain Crookes ATCC 8739	Anaerobic	MB	106.6	93.6	38.9	0.0
		BV	98.0	84.8	25.2	0.0
	Aerobic	MB	62.4	62.3	3.0	0.0
		BV	0.0	0.0	0.0	0.0
Strain B ATCC 11303	Anaerobic	MB	42.0	39.5	18.4	0.0
		BV	45.2	40.5	18.9	0.0
	Aerobic	MB	36.0	32.4	1.3	0.0
		BV	0.0	0.0	0.0	0.0
University of Washington strain 325b	Anaerobic	MB	90.6	83.1	40.8	0.0
		BV	80.2	74.2	36.5	0.0
	Aerobic	MB	77.1	73.6	3.0	0.0
		BV	0.0	0.0	0.0	0.0
University of Illinois strain K-12 (58-161)	Anaerobic	MB	50.1	44.0	21.6	0.0
		BV	47.7	42.6	20.1	0.0
	Aerobic	MB	47.5	43.5	1.5	0.0
		BV	0.0	0.0	0.0	0.0

[a] Cells grown aerobically at 30 C on a reciprocating platform shaker or anaerobically at 37 C in stationary culture in a defined medium supplemented with selenium compounds as indicated. Abbreviations: MB, methylene blue; BV, benzyl viologen.

electron acceptor. Very little enzyme activity could be detected from cells grown in the medium with selenomethionine. Regardless of the presence or absence of selenium compounds, aerobic cultures did not exhibit enzyme activity when the electron acceptor used for the assay was benzyl viologen. Like the anaerobic cultures, the aerobic cultures from medium deprived of selenium had no detectable enzyme activity. These results may suggest that the mechanism for the biosynthesis of formic dehydrogenase differs between aerobically and anaerobically grown *E. coli*. However, the presence of two different formic dehydrogenases as suggested by other workers (2) may also be a possibility.

In an attempt to demonstrate the relationship between formic dehydrogenase and selenium, radioactive selenium-75 (approximately 1,500 counts per min per ml, final concentration) as selenous acid in 0.7 M HCl (New England Nuclear, Boston, Mass.) was added to the defined medium in addition to 1.0 μg of sodium selenite per 100 ml of growth medium. A 25% (w/v) cell suspension of aerobically grown *E. coli* strain Crookes (ATCC 8739) in 0.05 M potassium phosphate buffer, *p*H 6.8, was disrupted with a Branson LS-75 sonifier having a 0.5-inch tip. Sonic treatment at 20 kc was carried out in a succession of 15-sec exposures to a total of 8 min. Cell debris and intact cells were removed by centrifugation at 10,000 × *g* for 30 min. The cell-free extract was again centrifuged at 110,000 × *g* for 1 hr to obtain the particulate fraction as the pellet which contained most of the enzyme activity. Solubilization and purification of particulate formic dehydrogenase was followed by the scheme of Linnane and Wrigley (5). The solubilized enzyme was then adsorbed to and eluted from freshly prepared calcium phosphate gel as described by Itagaki et al. (3). The gel eluate was further purified by layering on a sucrose gradient of 15 to 50%, made in 0.05 M potassium phosphate buffer, (*p*H 6.8), and centrifuging for 11 hr in a Spinco SW39 rotor at 170,000 × *g*. Fractions were collected by gravity and assayed for protein, radioactivity, and enzyme activity. The total protein content of each fraction collected was determined by the method of Lowry et al. (6). The radioactive selenium-75 in each fraction was determined in a 1.5 by 2 inch (approximately 3.8 by 5 cm) sodium iodide scintillation well counter (Picker) which served as a gamma-ray detector. Pulses were amplified and passed through a single-channel analyzer (Picker model 5811) set to pass pulses arising from 120 to 280 kev

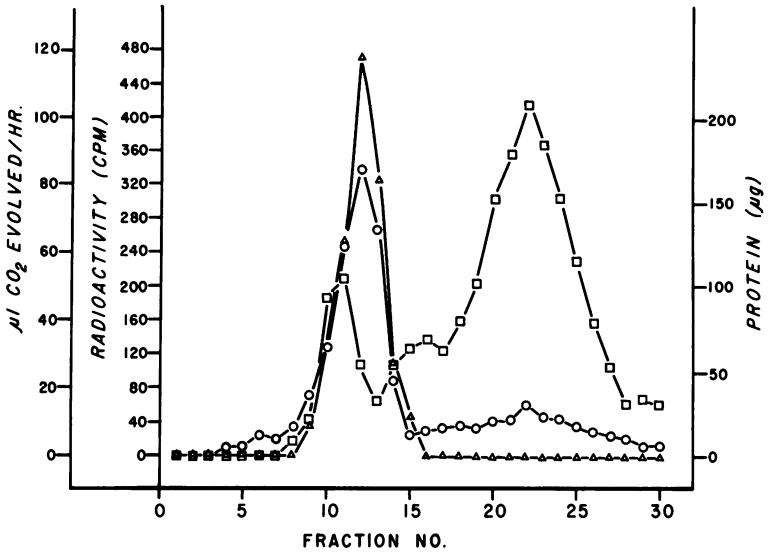

FIG. 1. *Sucrose gradient centrifugation of gel eluate from E. coli strain Crookes (ATCC 8739). Each fraction collected was diluted to 2.0 ml with 0.05 M potassium phosphate buffer, pH 6.8, and assayed for radioactivity* (○), *protein* (□), *and formic dehydrogenase activity* (△) *with methylene blue used as electron acceptor.*

TABLE 2. *Coincidence of selenium-75 incorporation and formic dehydrogenase activity in cell-free preparations of E. coli strain Crookes (ATCC 8739)*

Fraction	Microliters of CO_2 evolved per min per mg of protein	Counts per min per mg of protein
Particulate fraction	2.2	200
Gel eluate	16.8	1,040
Sucrose gradient peak fraction	40.0	6,476

gamma rays. The output pulses from the analyzer were counted in a scaler (Picker model 5810) for periods from 5 to 10 min.

The results are presented in Fig. 1. The radioactivity peak and the enzyme activity peak overlapped one another and lay close to the small protein peak. Radioactivity in the large protein peak is very low and virtually no detectable enzyme activity was seen. The coincidence of selenium-75 incorporation and formic dehydrogenase activity in cell-free preparations of *E. coli* (Fig. 1 and Table 2) suggests the presence of selenium in the enzyme since the peak fraction from the sucrose gradient showed an increase of 2.4-fold and 6.2-fold in enzyme specific activity and specific radioactivity, respectively, from the gel eluate.

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