Note

Assimilation of Elemental Sulfur by a Mutant of Escherichia coli B

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In most microorganisms, L-cysteine is synthesized via a sulfate reduction system as shown in Scheme 1.1 Detailed studies on this system have been carried out with E. coli, a typical heterotrophic bacterium. This system has been proven to be an assimilative process for sulfur during the biosynthesis of sulfur-containing amino acids.

In the course of our studies on the biosynthesis of sulfur-containing amino acids, we found that one of the mutants derived from E. coli B could utilize elemental sulfur (S°) for growth. The utilization of S° has been demonstrated only in sulfur-bacteria such as Thiobacillus species. However, the utilization of S° by these bacteria was primarily for the dissimilatory metabolism of sulfur as shown by Murr et al.2 and Postage3 and there is no information on the assimilation of S° by heterotrophic bacteria. Here, we describe the growth on S° of E. coli B.

For the study of growth on S°, two strains (355 and M71) were used. 355 was the wild type strain of E. coli B. M71 was a double mutant having phenotypic characters of cysteine-requirement and methylglyoxal (1.4 mM) resistance. A cysteine-requiring mutant was obtained by N-methyl-N'-nitro-N-nitrosoguanidine treatment by the method of Adelberg et al.4 from strain 355. Methylglyoxal (1.4 mM) resistance was further added to this mutant by the method of Murata et al.5

These two strains were grown in 3.0 ml of basal medium (comprised of 0.7% K2HPO4, 0.3% KH2PO4, 0.1% NH4Cl, 0.01% MgCl2·6H2O and 0.5% glucose) with various supplementations of sulfur compounds. Incubation was carried out at 30°C with reciprocal shaking. All of the sulfur compounds except for S° (purity, 99.999%) were used after sterilization by millipore filtration. S° was added directly to the medium and autoclaved at 120°C for 15 min. S° was also used after washing with sterilized water. The growth was monitored by measuring the absorbance at 660 nm.

Figure 1 shows the growth of M71 and 355 on the medium containing various sulfur compounds. M71 could grow on the medium containing S°, though 355, a methylglyoxal sensitive strain, could not. M71 also could grow on the medium containing various sulfur compounds other than sulfate.

No growth of 355 on S° indicated that this strain could not incorporate S° into cells through the cell membrane. Therefore, the growth of M71 on S° seemed to be due to the altered permeability of the cell membrane caused by the mutation to methylglyoxal resistance, since methylglyoxal is thought to cause a drastic change in cell membranes.5,6

Inability of M71 to grow on sulfate indicated that the cysteine requirement was presumably due to the loss of the ability to reduce sulfate. The inefficient utilization of sulfite by M71 seemed to be due to the low activity of sulfite reductase. In fact, this enzyme activity for M71 was about 1/4 of that of 355 (data are not shown).

Figure 2 shows the utilization of sulfur compounds. The minimal amount of S° (10 μM) required for the growth of M71 was almost the same as that in the case of other sulfur compounds. Judging from the purity of the S° used, this result indicated that M71 was utilizing S° for the synthesis of sulfur-containing organic compounds.

Fig. 1. Growth of 355 (Left) and M71 (Right) on Various Sulfur Compounds.

355 and M71 were grown in media containing various sulfur compounds at a concentration of 100 μM. Symbols: (●), elemental sulfur (S°); (○), Na2S; (□), Na2S2O3; (△), L-cysteine; (○), Na2SO3; (▲), Na2SO4.

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Fig. 2. Effect of Various Sulfur Compounds on the Growth of M71.

Cultivation was carried out at 30°C for 24 hr with supplementation of sulfur compounds. Symbols: (●), elemental sulfur (S°); (○), Na₂S; (□), Na₂S₂O₃; (△), l-cysteine; (□), Na₂SO₃; (▲), Na₂SO₄.

As can be seen in Figs. 1 and 2, thiosulfate and sulfide were good sulfur sources for M71, indicating that S° may be converted to thiosulfate and then to sulfide and sulfite. In fact, the accumulation of thiosulfate was found in the culture fluid of M71. Yagi et al.¹) and Wainwright et al.²) also demonstrated the oxidation of S° to thiosulfate by Streptomyces species and Fusarium solani, respectively. The direct reduction of S° to sulfide (dotted line in Scheme 2) was also likely to occur, but this reduction is found only in sulfate-reducing bacteria such as Desulfovibrio species³,⁴) and has not been found in heterotrophic bacteria such as E. coli B. Thus, we postulate the pathway for S° utilization by M71 to be as follows (Scheme 2).

The utilization of S° by E. coli B is of great interest for the biosynthesis of sulfur-containing amino acids. We are now investigating in detail the scheme of S° assimilation and have constructed a strain of E. coli B having high sulfite-reducing activity.

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REFERENCES