Effect of Selenomethionine on Growth of *Escherichia coli* and *Bacillus megaterium*

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Studies with methionineless strains of *Escherichia coli* WWU and *Bacillus megaterium* KM have shown that selenomethionine only partially satisfies their methionine requirement.

This study was based upon the report (2) that selenomethionine (Se-M) could completely replace methionine (S-M) for the normal exponential growth of *Escherichia coli* ML-30. The growth studies reported here were preliminary to experiments designed to determine whether the methyl group of Se-M could serve as an effective methyl donor for nucleic acids.

Bacillus megaterium KM: thymine⁻ methionine⁻ (KM:T⁻ M⁻) has been described previously (4). *E. coli* WWU, a polyauxotrophic derivative of *E. coli* 15 T⁻ with a block in the methionine biosynthetic pathway (1), was obtained from R. C. Bockrath of Indiana University, Indianapolis. Both organisms were grown in a basal medium consisting of 1.0% glucose, 0.65% sodium L-glutamate, 0.3% K₂HPO₄, 0.1% KH₂PO₄, 0.1% Na₂SO₄, 0.005% MgSO₄, 3 × 10⁻⁴ M ferric citrate, 1 to 5 × 10⁻⁴ M thymine, and 5 × 10⁻⁴ M DL-methionine (final pH 7.0). For the growth of *E. coli* WWU, the basal medium was supplemented with 0.038 M NH₄Cl, and uracil, L-arginine, L-proline, and L-tryptophan at a final concentration of 5 × 10⁻⁴ M each. Cells were grown at 37 C with vigorous aeration. Turbidity was determined with a Klett-Summerson colorimeter (filter no. 64). In each experiment, exponentially growing cells were harvested by centrifugation at 0 C, washed twice with cold, unsupplemented basal medium (minus thymine and methionine), and finally resuspended at about one-tenth cell density in the appropriately supplemented basal medium.

For viable cell counts, suspensions were diluted in the basal medium, and 0.05-ml samples were spread on the surface of plates containing tryptose-yeast extract agar (3) supplemented with 5 × 10⁻⁴ M thymine and 5 × 10⁻⁴ M DL-methionine. Plates

were incubated for 24 hr at 37 C prior to counting colonies.

Photographs were taken with a Bausch and Lomb binocular phase-contrast microscope equipped with a Polaroid camera attachment and a flat-field oil immersion objective lens (100X; 1.25 N.A.). A droplet of cell suspension was placed on a slide containing a thin layer of 1% Ionagar no. 2 (Consolidated Laboratories, Inc., Chicago Heights, Ill.), covered with a cover slip, and photographed with Polaroid black and white, 300 speed/type 107 film.

All compounds were commercial samples of analytical grade. DL-Selenomethionine was obtained from K & K Laboratories, Inc., Plainview, N.Y.

Figure 1 shows the comparative abilities of Se-M to substitute for S-M in methionineless strains of *E. coli* and *B. megaterium*. It can be seen that at equivalent concentrations, the maximum viable count achieved with Se-M is considerably less than that with S-M. Also, in general, S-M supports a more rapid rate of growth than Se-M. In several experiments with Se-M, maximum cell densities of *E. coli* were obtained with 10⁻⁴ M Se-M, whereas with *B. megaterium* 5 × 10⁻⁵ M Se-M was optimal. As shown in Figure 1B, the viable count of *B. megaterium* increases approximately fourfold over a 2-hr period of amino acid starvation and then reaches a plateau. This is in contrast to the situation with *E. coli* WWU (Fig. 1A), and could be explained by the presence of a substantial intracellular methionine pool or by the fragmentation of cell chains. When *E. coli* WWU (starting with approximately 10⁸ viable cells/ml) was subcultured in media containing 10⁻⁴ M Se-M in place of S-M, there was a progressive decrease in the growth rate, with a cessation of growth after about five doublings. Continued incubation resulted in the selection of methionine⁺ revertants. In a similar experiment

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FIG. 1. Comparative effects of DL-methionine (S-M) and DL-selenomethionine (Se-M) on viability of (A) *E. coli* and (B) *B. megaterium*. Methionine-grown cells were harvested and washed as described. Cells were suspended in the complete basal medium in the absence of methionine and were shaken for 1 hr at 37 C to deplete endogenous methionine pools. Additions were made at the times indicated by the arrow.

with *B. megaterium* (starting with approximately 10^7 to 2×10^7 viable cells/ml), growth ceased after about three doublings in the presence of 5×10^{-5} M Se-M. This is in contrast to a methionineless strain of *E. coli* ML-30 which has been reported to grow exponentially through more than 100 generations in a minimal liquid containing 10^{-4} M Se-M in place of S-M (2); the generation time with Se-M was found to be about 30% greater than that with S-M. Thus, both *E. coli* WWU and *B. megaterium* KM differ from *E. coli* ML-30 in that they can use Se-M in place of S-M for only a very limited number of doublings.

The ability of Se-M to support thymineless death is shown in Fig. 2. It can be seen that for the first 2 hr both Se-M and S-M support similar

rates of thymineless death in *E. coli* (Fig. 2A). With *B. megaterium*, on the other hand (Fig. 2B), Se-M is a poor substitute for S-M. Notice that double starvation (minus thymine and methionine) is not lethal for *B. megaterium* but is lethal for *E. coli*.

When *E. coli* WWU is grown with 10^{-4} M Se-M instead of S-M for about five doublings, the normally rod-shaped cells are converted to spherical or oval forms (Fig. 3). Exposure of *B. megaterium* to 5×10^{-5} M Se-M does not result in any morphological change. In an experiment involving the preparation of cell extracts from *E. coli* WWU, it was found that although the rod-shaped cells are lysed by exposure to lysozyme-Versene, the spherical or oval cells are resistant to lysis.

FIG. 2. Comparative abilities of DL-methionine (S-M) and DL-selenomethionine (Se-M) to support thymineless death in (A) *E. coli* and (B) *B. megaterium*. Methionine-grown cells were harvested and washed as described. Cells were suspended in the complete basal medium in the absence of thymine and methionine and the above additions were made at zero time. *E. coli* = 1×10^{-4} M S-M or Se-M. *B. megaterium* = 5×10^{-5} M S-M or Se-M. Incubation was at 37 C.

FIG. 3. Morphology of *E. coli* WWU grown in basal medium containing (A) 1×10^{-4} M DL-methionine and (B) 1×10^{-4} M DL-selenomethionine. Final magnification, approximately 1,800X.

FIG. 2

FIG. 3

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