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Utilization of D-Methionine by *Escherichia coli*

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ABSTRACT

COOPER, STEPHEN (University Institute of Microbiology, Copenhagen, Denmark). Utilization of D-methionine by *Escherichia coli*. J. Bacteriol. **92**:328–332. 1966.—Methionine-requiring strains of *Escherichia coli* grow on D-methionine. Mutants can be isolated which cannot grow on D-methionine. The D-methionine nonutilizing mutation is independent of the methionine requirement, and maps near the *lac* region of the *E. coli* genome. Growth of methionine-requiring strains on D-methionine is dependent upon aerobic conditions. Cells grown on D-methionine have a sixfold greater ability to incorporate D-methionine into protein than cells grown on L-methionine. The incorporation of D-methionine is inhibited by L-methionine.

It has been noted that methionine-requiring strains of *Escherichia coli* can utilize D-methionine for growth (2, 4). A detailed study of the mechanism by which D-methionine is utilized has not been presented. This paper details the results of studies with whole cells on the mechanism, genetics, and physiology of D-methionine utilization.

MATERIALS AND METHODS

Chemicals. Keto-methionine was the generous gift of Alton Meister. Radioactive D- and L-methionine (labeled with C^{14} in the methyl group) and unlabeled D- and L-methionine were purchased from Calbiochem.

Bacterial strains. Strains K-12 and TAU/M^- were obtained from O. Maaløe. Strain W7, obtained from Yaakov Aharonovitch, is a methionine- or B_{12}-requiring auxotroph which is unable to methylate homocysteine. Strain P4X is a male strain of *E. coli* which requires methionine and thiamine. *E. coli* male strain Hfr H (3) was obtained from William Hayes. Additional strains were made by mating and mutation as described in Fig. 1.

Media. Minimal tris(hydroxymethyl)aminomethane (Tris)-buffered media was prepared according to Maaløe and Hanawalt (5). Uracil and amino acids other than methionine were usually supplied at a concentration of 100 μg/ml and thymine at 10 μg/ml. Methionine for growth studies was present at a concentration of 50 or 100 μg/ml. Limiting glucose for the preparation of overnight cultures was 0.02% glucose, and glucose for unlimited growth was 0.2% glucose.

Measurements of bacterial growth and yield. Bacterial density was measured spectrophotometrically at 450 mμ (OD_{450}) in a Zeiss PMQII spectrophotometer. Bacteria were grown in logarithmic phase by bubbling air through the culture. Yields of bacteria grown on different concentrations of methionine were measured after shaking the cultures in cotton-stoppered Erlenmeyer flasks at 37 C. Anaerobic conditions were produced by autoclaving media in small screw-cap bottles. The media filled the entire bottle, and, after rapid cooling and inoculation, the bottle was sealed.

Isolation of mutants and mapping of mutations. Mutants unable to grow on D-methionine were isolated by use of a penicillin enrichment procedure (1). Mutations were produced with ultraviolet irradiation. Mutants unable to grow on D-methionine (D^-) were selected from ultraviolet-irradiated methionine-requiring strains, by replicating the colonies (which were grown on minimal media containing L-methionine) onto plates which contained approximately 2.5 μg of D-methionine per ml. Mapping of the D^- mutants was performed by the blender technique (3). Methionine-requiring strains which are made prototrophic for methionine have been designated M^r to signify that the lack of a methionine requirement has been produced by reversion of an auxotroph. It is not known whether the reversion is at the same or another locus.

Incorporation of radioactive D- or L-methionine. Bacteria were grown overnight in the media indicated with limiting glucose (0.02%); then excess glucose (0.2%) was added, and the cells were incubated with aeration for at least 1 hr. The cells were filtered and washed on membrane filters, and were resuspended in fresh prewarmed media lacking methionine. The density of the cells was adjusted to an optical density at 450 mμ (OD_{450}) of 0.3 to 0.5, and the cells were immediately pipetted into tubes containing the radioactive amino acids. Samples were taken at various times and were precipitated and extracted with 5% trichloroacetic acid at 88 to 92 C for 15 min. After cooling, the precipitates were filtered on membrane filters, dried, and counted in a Nuclear-Chicago scintillation counter.

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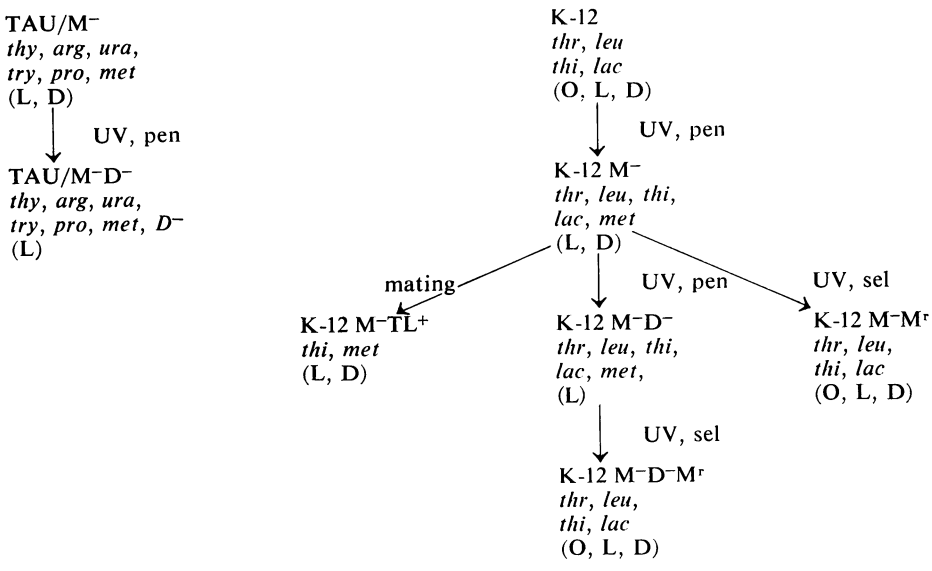

FIG. 1. *Isolation scheme, genotype, and phenotype of strains used in the experiments reported. Normal mutant designations are used except that D^- stands for inability to grow on* D*-methionine (or, in the case of a methionine prototroph, inability to incorporate radioactive* D*-methionine), and M^r stands for a reversion of a methionine requirement to methionine independence. This designation is used to show the history of a strain because a D^- mutation can only be selected for in a methionine-requiring bacterium. The symbols in parentheses below each strain description indicate the methionine medium which will allow growth of a particular strain, O, L, and D, standing for no methionine,* L*-methionine, and* D*-methionine, respectively. Note that while strains K-12 M^-M^r and K-12 $M^-D^-M^r$ are phenotypically identical with regard to their methionine requirements, only K-12 M^-M^r will incorporate radioactive* D*-methionine. UV stands for ultraviolet irradiation used as a mutagen, pen means enrichment by penicillin treatment and selection by replica plating, and sel means selection for prototrophs on plates with no added methionine. Italics indicate nutrient requirements.*

RESULTS

Growth on D*-methionine.* All methionine-requiring strains of *E. coli* tested grew on D-methionine. They grow slightly slower on D-methionine compared with the rate of growth on L-methionine, as shown in Table 1. The yield of bacterial mass, however, is identical (Fig. 2). Accurate measurements have revealed no significant difference in the mass of bacteria (measured spectrophotometrically at 450 mμ) when grown on D- or L-methionine.

The results presented in Fig. 2 are at variance with the results of Lampen, Jones, and Perkins (4), who found that the growth on D-methionine was considerably less than the growth on L-methionine. They postulated that some factor was limiting. However, they could not determine what it was. The results presented in Table 2 can explain the difference in results. Methionine-requiring strains in D-methionine-containing medium require oxygen for growth (Table 2). The results in Fig. 2 were made with cultures continuously shaken. Results identical to those of Lampen, Jones, and Perkins can be obtained if the same experiment described in Fig. 2 is performed in test tubes with caps or cotton plugs. It appears, therefore, that the limiting component in the experiments of Lampen, Jones, and Perkins (4) was oxygen, and the culture stopped growing when the residual oxygen in the medium was depleted by the growing bacteria. *E. coli* thus appears to be an obligate aerobe when methionine-requiring strains are grown on D-methionine.

TABLE 1. *Growth rates of methionine-requiring Escherichia coli strains on* L*- and* D*-methionine*

Strain	L-Methionine	D-Methionine
K-12 M⁻	53[a]	83
P4X	40	65
TAU/M⁻	40	44
W7	38	63

[a] Rates indicated in minutes required for doubling.

Isolation of mutants unable to grow on D*-methionine.* Mutants unable to utilize D-methionine were isolated from methionine-requiring strains of *E. coli* as indicated in Fig. 1. Two separate strains

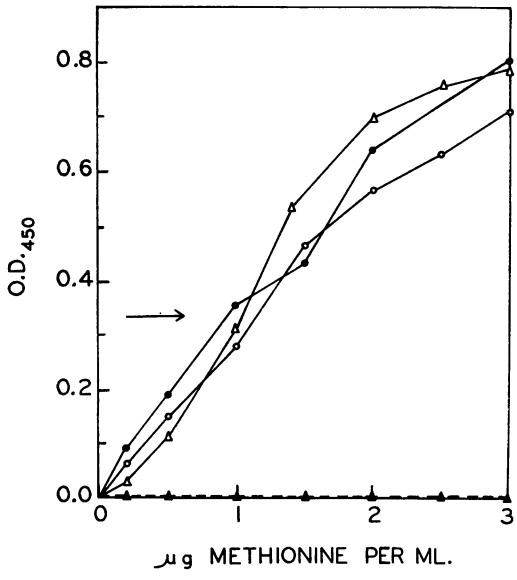

FIG. 2. *Yield of bacteria on various concentrations of* D- *or* L-*methionine. TAU/M⁻ or TAU/M⁻D⁻ cells were grown in cotton-stoppered Erlenmeyer flasks with shaking in a warm room for 20 hr. Similar results are obtained with mutants of the Escherichia coli K-12 strains. Symbols;* ○, *TAU/M⁻ on* L-*methionine;* ●, *TAU/M⁻ on* D-*methionine;* △, *TAU/M⁻D⁻ on* L-*methionine;* ▲, *TAU/M⁻D⁻ on* D-*methionine. Arrow indicates yield of TAU/M⁻D⁻ on 1,000 μg/ml of* D-*methionine.*

TABLE 2. *Growth of Escherichia coli on* D- *and* L-*methionine under aerobic and anaerobic conditions*

Methionine	Aerobic[a]		Anaerobic	
	M+[b]	M−	M+	M−
D-Methionine	+[c]	+	+	−
L-Methionine	+	+	+	+
D-Methionine and L-methionine	+	+	+	+
No methionine	+	−	+	−

[a] Conditions described in Materials and Methods.

[b] M+ is K-12 and M− is K-12 M⁻. These results have also been observed in other strains tested.

[c] Symbols: +, growth (turbid); −, no growth (faint turbidity).

were used. The D⁻ mutants were easy to produce; both were obtained on the first screening. They are quite stable, and not "leaky," since growth on high (100 μg/ml) concentrations of D-methionine for extended periods (1 week) did not lead to any growth beyond that attributable to contamination of the D-methionine with L-methionine. It can be seen from the results in Fig. 2 that commercial D-methionine allows growth of a D⁻ strain with an efficiency of 0.1%. This can be explained by a contamination of D-methionine with 0.1% L-methionine. In the results described below, the experiments were performed with the bacteria derived from strain K-12.

As indicated in Fig. 1, the methionine-requiring strain and the methionine-requiring, D-methionine-nonutilizing strain can both be reverted by ultraviolet irradiation to obtain methionine prototrophs by selection on methionine-deficient plates. The two strains, K-12 M⁻M^r and K-12 M⁻D⁻M^r are both phenotypically identical by all growth criteria. However, only the former will still incorporate radioactive D-methionine. This result indicates that the mutation leading to D⁻ cells is not dependent on the presence of a methionine requirement, although the D⁻ mutation can only be isolated in a strain which does require methionine.

Both the D-methionine-utilizing and D-methionine-nonutilizing strains of K-12 will grow on keto-methionine.

Mapping of the D mutation. The D⁻ mutant of strain K-12 which required threonine and leucine and was unable to ferment lactose was mated with a male K-12 strain, Hfr H. The female was streptomycin-resistant and the male was streptomycin-sensitive. Selection was made on minimal plates containing streptomycin and missing the pertinent supplement. The times of entry of the various loci are: *thr* and *leu*, 9 min; D⁻ (D-methionine utilization), 19 min; and *lac*, 21 min. It appears that one locus of D-methionine utilization is approximately 2 min away from the *lac* region toward threonine and leucine. Other mutants were not prepared, and therefore it is not known whether other D⁻ mutants would map at other loci. The locus near the *lac* region is not near any known methionine loci (7).

Incorporation of radioactive D-*methionine or* L-*methionine into E. coli.* Cells grown on D- or L-methionine were tested for their ability to incorporate radioactive D- or L-methionine into hot trichloroacetic acid-precipitable material (presumably bacterial protein). As can be seen in Fig. 3, the ability of *E. coli* to incorporate the radioactivity from D-methionine is "induced" by growth on D-methionine (or, conversely, "repressed" by growth on L-methionine). At present, however, no distinction between "induction" by D-methionine and "repression" by L-methionine can be made. Growth of cells in threonine and leucine increases approximately threefold the ability of cells grown on L-methionine to incorporate D-methionine, thus reducing the observed stimulation by growth in D-methionine to a twofold increase (over incorporation by cells grown in the presence of L-

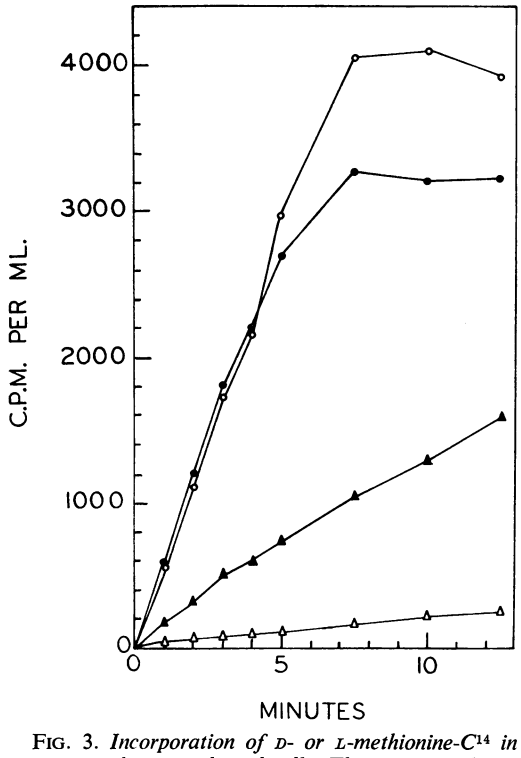

FIG. 3. *Incorporation of* D- *or* L-*methionine-C*^{14} *in* D- *or* L-*methionine-adapted cells. The strain used was K-12 M*$^-$ *TL*$^+$*, prepared as described in Fig. 1.* L-*Methionine-adapted bacteria were present at an optical density at 450 mμ of 0.410, and* D-*methionine-adapted cells were present at an optical density at 450 mμ of 0.400. Concentrations of* D- *or* L-*methionine were 0.1 μg/ml. Symbols;* ●, D-*methionine-adapted cells incorporating* L-*methionine;* ○, L-*methionine-adapted cells incorporating* L-*methionine;* ▲, D-*methionine-adapted cells incorporating* D-*methionine;* △, L-*methionine-adapted cells incorporating* D-*methionine.*

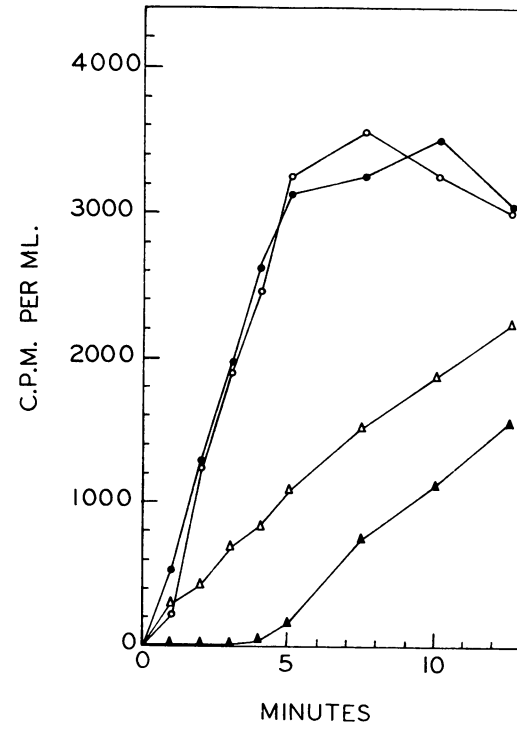

FIG. 4. *Incorporation of* D- *or* L-*methionine into* D-*methionine-adapted cells in the presence or absence of unlabeled* D- *or* L-*methionine. All amino acid concentrations were 0.1 μg/ml. The bacterial strain used was K-12 M*$^-$*, at an optical density at 450 mμ of 0.376. Symbols:* ●, L-*methionine-C*^{14}*;* ○, L-*methionine-C*^{14} *and unlabeled* D-*methionine;* △, D-*methionine-C*^{14}*;* ▲, D-*methionine-C*^{14} *and unlabeled* L-*methionine.*

methionine). The mechanism of this effect is not known, nor is the mechanism which increases the ability of D-methionine-grown cells to incorporate D-methionine.

L-Methionine eliminates the ability of D-methionine-adapted cells to incorporate radioactive D-methionine, until that time when the L-methionine is depleted by incorporation into protein (Fig. 4). Further, D-methionine does not dilute out the incorporation of radioactive L-methionine (Fig. 4). When 100 μg/ml of D-methionine and 0.1 μg/ml of radioactive L-methionine were added to cells, the rate of incorporation of L-methionine was only half of what it would have been in the absence of D-methionine. This is to be expected from the fact that D-methionine is contaminated to the extent of 0.1% with L-methionine. Only the dilution of radioactive L-methionine with the added L-methionine leads to a reduction in the rate of incorporation. The results suggest an actual inhibition of the conversion of D-methionine to L-methionine, for, if the conversion was not inhibited, a decrease in the rate of incorporation of radioactive L-methionine due to a dilution of the L-methionine with nonradioactive L-methionine would be observed. Also, if there was no inhibition, a slow incorporation of radioactivity from D-methionine would take place in the presence of L-methionine. Thus, a 500-fold excess of D-methionine does not compete to any measureable extent with the incorporation of labeled L-methionine. This result is formally similar to "feedback inhibition" of the D-methionine-utilizing system. The mechanism of this inhibition of D-methionine utilization by L-methionine is not known.

DISCUSSION

The ease with which one can obtain mutants unable to grow on D-methionine suggests that there is only one pathway for the conversion of D- to L-methionine. If there were two or more alternate pathways, it would probably be difficult to pick up mutants unable to grow on D-methio-

nine, for a double mutant would have to be produced. One question which is of interest regarding the utilization of D-methionine by *E. coli* is whether the mechanism for the conversion of D- to L-methionine is specifically adapted for this purpose, or whether the conversion is due to activities of some other enzyme or enzymes which normally function in some other pathway. The experiments presented in this paper, indicating that the utilization of D-methionine is controlled by the presence of D- or L-methionine, suggest that the conversion mechanism is specifically adapted for the utilization of D-methionine.

The similarity of yield of bacteria grown on limiting amounts of D- or L-methionine implies that there cannot be any major metabolic conversions of D-methionine unless the ultimate product is L-methionine. If there were to occur any major conversion of D-methionine to any product which was not ultimately L-methionine, then the yields of bacteria on D-methionine would be less than on L-methionine. This conclusion, coupled with the conclusion that there is one pathway for the conversion of D- to L-methionine suggests that any conversion of D-methionine found in cell-free extracts is probably on the pathway to L-methionine. In extracts of *E. coli*, there is a D-methionine deaminating activity producing keto-methionine from D-methionine (Cooper, *unpublished data*). If the above reasoning is correct, one may postulate the following mechanism for the conversion of D- to L-methionine:

$$\text{D-methionine} \xrightarrow{O_2} \text{keto-methionine} \xrightarrow{NH_2\ \text{donor}} \text{L-methionine}$$

This mechanism postulates an oxidative deamination of D-methionine to yield keto-methionine, and a transamination to yield L-methionine. The existence of the first step is consistent with the results, indicating that the growth of *E. coli* on D-methionine is dependent upon aerobic conditions. The second step is feasible, because methionineless *E. coli* grow on keto-methionine even though the normal pathway of methionine synthesis does not involve this as an intermediate. Rudman and Meister (6) have shown that *E. coli* cells contain methionine-α-ketoglutarate transaminating activity.

Three other points may be noted regarding the observations noted in this paper. First, the location of the D^- locus may be of value as it is near the *lac* region and may be a good marker for various genetic studies. Second, the mutant unable to grow on D-methionine is a good bioassay organism for L-methionine, and the difference in growth between the D-methionine-utilizing and -nonutilizing strains can be used as a measure of D-methionine. Third, the slow growth on D-methionine compared with rapid growth on L-methionine suggests that the conversion of D- to L-methionine is the rate-limiting reaction when the cells are grown on D-methionine. This implies that a "slow feed" of L-methionine is probably occurring, which may be of value in producing a derepression of some methionine-synthesizing enzymes in methionine auxotrophs.

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