サルモネラおよび枯草菌の自然突然変異に対するL-エチオニンの抗変異効果

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著者	井上, 正 鈴木, 音哉 賀田, 恒夫
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Bio-antimutagenic effect of L-ethionine on the spontaneous mutagenesis in *Salmonella* and *Bacillus subtilis*

BY Tadashi INOUE*, Otoya SUZUKI*[1)], Tsuneo KADA* and Taro FUJII**

**Laboratory of Mutagenesis and* ***Genetic Stock Research Center, National Institute of Genetics, Mishima, Shizuoka-ken* 411

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ABSTRACT

L-ethionine, a hepatocarcinogen, exhibited a strong bio-antimutagenic effect against spontaneous mutation in Ames' *Salmonella* strains as well as in a *Bacillus subtilis* mutator strain whose mutator activity was due to an error-prone DNA replicating enzyme. The bio-antimutagenic effect of L-ethionine was completely abolished by the presence of L-methionine, but not by D-methionine, suggesting that genetic effect of L-ethionine was expressed through its interaction with L-methionine metabolism in the treated cells.

1. INTRODUCTION

Ethionine, the ethyl analogue of the essential amino acid methionine, is one of the well-known hepatocarcinogen in rat (Farber 1963). However, it dose not bind to DNA and appears to be non-mutagenic in the Ames' *Salmonella* tester strains (McCann *et al.* 1975) although it exhibits mutagenicity in *Coprinus lagopus* (Talmud and Lewis 1974), soy beans, (Fujii 1981) and *Escherichia coli* (Zgaga 1986). Weisner and Troll (1981) found that ethionine blocked certain SOS functions including prophage induction and error-prone DNA repair activity in the thermally induced *tif* mutant of *E. coli* B/r, suggesting that ethionine may interfere with the function of RecA protein in *E. coli*. Barbe *et al.* (1984) also presented data suggesting a possible interference of ethionine with RecA441 protein of *E. coli* K12. More redently, Poder *et al.* (1983) reported that mutagenesis in *Salmonella* by 9-aminoacrydine but not by 2-aminopurine was abolished by ethionine. They suggested that ethionine inhibited a repair system which removes and/or eliminates mismatched bases. These data, though somehow contradictory to each other, suggest that ethionine interferes with DNA metabolism resulting in an alteration of mutation frequency of the treated cells.

In order to elucidate the molecular mechanisms, we examined the genetic effect of ethionine using bacterial systems which exhibit high spontaneous mutation frequency due to known molecular mechanisms.

[1)] Present address: Sogo Biomedical Labs. Ltd. Kawagoe-shi, Saitama-ken 350, Japan.

2. MATERIALS AND METHODS

The *Salmonella* tester strains, TA98, TA100, TA1535 and TA1538 were kindly donated by Prof. B. N. Ames of the Univ. of California. We selected and used bacterial clones with relatively high spontaneous mutation frequency for easier detection of the reduction of the spontaneous mutation. *Bacillus subtilis* NIG1125 (*his met mut-1*) has been described in a previous paper (Inoue *et al.* 1981).

Plate incorporation assays with the *Salmonella* tester strains were carried out according to the methods of Ames *et al.* (1975) using a semi-enriched-agar plate (MB plate; Inoue *et al.* 1981) supplemented with 0.1 mg/ml of biotin. Chemicals including ethionine were incorporated into the top agar together with the bacteria. Concentration of chemicals is expressed as μg/ml in the plate. Double reversion of *B. subtilis* NIG1125 (*his met mut-1*) to prototroph was assayed on the MB agar plate as described previously (Inoue *et al.* 1981). In this case, ethionine was incorporated into hard agar. For the determination of viability, the cell suspension was appropriately diluted and was inoculated on the same MB plates. Plates were incubated at 37°C for 2 days prior to counting.

L-ethionine, D-ethionine, L-methionine and D-methionine were products of Nakarai Chemicals LTD, Kyoto. All other chemicals were reagent grade.

3. RESULTS

Ames' *Salmonella* tester strains TA98 and TA100 are known to exhibit relatively high spontaneous mutation rate compared to their parental strains, TA1538 and TA1535, due to the presence of plasmid pKM101. As shown in Table 1, the number of spontaneous mutations decreased depending on the concentration of L-ethionine incorporated in the plates. Very few revertant colonies were developed at 5 μg/ml of L-ethionine whereas D-ethionine did not exhibit such an antimutagenic effect. The reduction of the revertant colonies by L-ethionine is not due to the toxicity of this chemical, since the background lawns on plate with L-ethionine were not distinguishable from those without L-ethione. This was also confirmed by the fact that the viability of the cells was not affected at all at concentrations of up to 400 μg/ml (Table 2). Another explanation for the antimutagenic effect of L-ethionine is that this amino acid specifically inhibits the growth of histidine-prototrophs on the selection plates. However, this was not the case, since no difference was detected in the degree of sensitivity to L-ethionine among a number of spontaneously appearing His^+ clones and His^- parental clones (data not shown).

The relatively high spontaneous mutation rates in TA98 and TA100 are thought to be due to the plasmid harbored in these strains. We therefore

Table 1. *Effect of L- and D-ethionine on the spontaneous mutagenesis in Salmonella tester strains TA98 and TA100*

Strain	Concentration (μg/ml)		His⁺/plate
	L-ethionine	D-ethionine	
TA98	0	0	41
	1.0	0	27
	2.5	0	14
	5.0	0	2
	0	1.0	40
	0	2.5	41
	0	5.0	41
TA100	0	0	455
	1.0	0	224
	2.5	0	63
	5.0	0	5
	0	1.0	372
	0	2.5	363
	0	5.0	391

Histidine reverse mutation was determined as described in Materials and Methods. Each figure is the representative of three to four plates.

Table 2. *Effect of L-ethionine on the viability of Salmonella tester strains TA98 and TA100*

Concentration of L-ethionine (μg/ml)	Viable cells per ml ($\times 10^8$)	
	TA98	TA100
0	1.92 (100)	1.16 (100)
50	1.88 (98)	1.09 (94)
100	1.83 (95)	1.11 (96)
200	1.90 (99)	1.04 (89)
400	1.89 (98)	1.08 (93)

For the determination of viability, appropriately diluted bacterial suspensions were inoculated with the indicated concentration of L-ethionine. Numbers in the parentheses represent percentages of control.

examined whether the antimutagenic effect of L-ethionine is specific for the spontaneous mutation caused by the plasmid. As shown in Table 3, L-ethionine also abolished spontaneous mutations in the parental strains, TA1538 and TA1535, which do not harbor the plasmids, indicating that plasmid-mediated mutagenesis is not specifically involved in the expression of the bio-antimuta-

Table 3. *Effect of L-ethionine on the spontaneous mutagenesis in Salmonella tester strains TA1538 and TA1535*

Strain	Concentration of L-ethionine (μg/ml)	His$^+$/plate
TA1538	0	51
	1	58
	3	29
	5	21
	10	9
TA1535	0	36
	1	7
	3	1
	5	0
	10	0

Histidine reverse mutation was determined as described in Materials and Methods. Each figure is the representative of three to four plates.

genic activity of L-ethionine.

The work of Bazill and Gross (1973) indicated that the *mut-1* mutant of *B. subtilis* produces an altered DNA polymerase III which is a chromosome replicating enzyme of this bacterium, and therefore the mutator activity of this strain is ascribed to error-proneness of the DNA replicating enzyme. We thus examined the effect of L-ethionine on the spontaneonus mutation induction in the *mut-1* strain of *B. subtlis* NIG1125 (*mut-1 his met*). The results shown in Table 4 indicate that L-ethionine is also effective in this system suggesting possible involvement of the error-prone DNA replicating enzyme in the expression of the bio-antimutagenic activity of this amino acid.

Table 4. *Effect of L-ethionine on the spontaneous mutagenesis in B. subtilis NIG1125 (his met mut-1)*

Concentration of L-ethionine (μg/ml)	His$^+$Met$^+$/plate	Viable cells/ml ($\times 10^8$)	Mutation frequency ($\times 10^{-8}$)
5	124	2.60	477
0.5	40	2.15	186
1.0	23	1.95	118
2.5	15	2.21	68
5.0	9	2.33	39
10.0	7	2.06	34

Viability and His$^+$Met$^+$ double mutation were determined as described in Inoue *et al.* (1981).

One of the most probable targets of the action of L-ethionine is the metabolism of L-methionine in the cells. Ethionine may be incorporated into protein in place of methionine or these two amino acids may compete with each other in the process of methylation of biomolecules including proteins and nucleic acids. We therefore tested whether the bio-antimutagenic activity of L-ethionine is modified by the presence of methionine. A typical result is shown in Fig. 1. In this experiment, an increasing amount of methionine was added to the plate under the condition where spontaneous mutagenesis was almost completely blocked by the presence of L-ethionine. As clearly seen in this figure, development of the revertant colonies was restored depending on the amount of L-methionine added in the plate, reaching the level comparable with that of original spontaneous mutation. In contrast, D-methionine had little, if any, restorative effect. These data strongly suggest that the bio-antimutagenic effect of L-ethionine is expressed through its interaction with L-methionine metabolism in the cells.

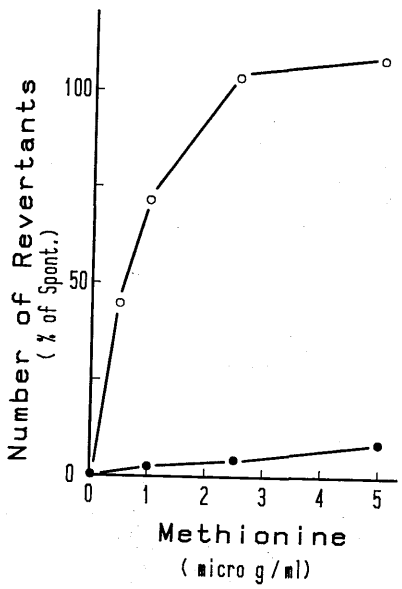

Fig. 1. Restorative effect of methionine on the L-ethionine-depressed spontaneous mutagenesis in *Salmonella* tester strain TA100. Indicated amount of L-methionine (closed circle) or D-methionine (open circle) was incorporated into the plate together with 5 μg/ml of L-ethionine. At this concentration of L-ethionine, few revertant oclo-nies appeared without methionine. The number of revertant colonies on the plate containing neither L-ethionine nor methionine was 275 in this experiment.

4. DISCUSSION

Ethionine has been known as a potent hepatocarcinogen in rats, but its mode of action during carcinogenesis has not yet been elucidated (see Farber

1963, for review). This amino acid has been also known as one of the typical carcinogens which are not mutagenic in Ames' tester strains. On the contrary, experimental results presented by Podger *et al.* (1983) and by Weisner and Troll (1981) as well as those of ours described in the present paper clearly show that ethionine is antimutagenic in certain bacterial mutation assay systems.

Among the possible ways by which L-ethionine exerts its antimutagenic effect are the inhibition of DNA methylation and the modification of protein. In the latter case, either the incorporation of ethionine into protein in place of methionine, or the inhibition of post-translational methylation may be involved. Weisner and Troll (1981) proposed a hypothesis that the function of RecA protein was impaired by the hypomethylation of specific sites in DNA in the ethionine-treated cells, resulting in the blockig of SOS functions including prophage induction and the inhibition of UV-induced mutation in *E. coli*. Our results do not necessarily support this hypothesis since spontaneous mutagenesis in the *mut-1* strain of *B. subtilis* which is caused by an error-prone DNA replicating enzyme and therefore is primarily irrelevant to the RecA-mediated mutagenesis was also abolished by L-ethionine (Table 4). It is also noteworthy that the antimutagenicity of L-ethionine was equally exhibited both in the strains harboring plasmid pKM101 and those do not. This fact implies that the expression of antimutagenicity is not directly relevant to the functions encoded by the plasmid in which the RecA-dependent induction of SOS functions are involved. Our experimental results suggest rather that the modification by L-ethionine of an enzyme which functions in repair/replication is a causal factor for the bio-antimutagenic action of this amino acid. In this context, the possible involvement of the chromosome roplicating enzyme, DNA polymerase III, in induced as well as spontaneous mutagenesis (Bridges and Mottershead, 1978: Bridges 1980) is full of suggestion. The proposal by Podger *et al.* (1983) that ethionine exerts its antimutagenicity through its interaction with a repair system is consistent with our results. Regardless of the exact mechanism, ethionine seems to have rather general genetic effect and may not only yield information on carcinogenesis but may also help to reveal the mechanisms for mutagenesis.

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