Note

Effect of S-9 Mix on Antimutagenic Activity of Autoxidized Linoleic Acid

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Received March 1, 1988

I have reported the antimutagenic effects of autoxidized linoleic and oleic acids on UV induced mutagenesis in *Escherichia coli* B/r WP2,¹⁾ but Yamaguchi *et al.* reported the mutagenic effect of autoxidized linolenic acid and linoleic acid on *salmonella typhimurium* TA 100 and TA 98.²⁾ They reported that mutagenic action appeared when these autoxidized acids were activated with S-9 Mix. They suggested that autoxidized fatty acids seem to be pro-mutagen and the ultimate mutagen is produced from reaction with S-9 Mix. Taking into account the antimutagenic activity of autoxidized linoleic acid, however, I suggest the possibility that S-9 Mix may be able to reduce the antimutagenic activity of autoxidized linoleic acid. The mutagenic action of autoxidized linoleic acid may be appear when the antimutagenic effect of autoxidized linoleic acid is reduced by S-9 Mix. For confirmation of these possibilities, I investigated the effects of S-9 Mix on the antimutagenic activity of autoxidized linoleic acid in UV induced mutagenesis of *E. coli* B/r WP2, and the AF2 induced mutagenesis of *S. typhimurium* TA 98.

Linoleic acid was obtained from Wako Pure Chemical Industries, Osaka. AF2 was obtained from Ueno Seiyaku, Osaka. S-9 was purchased from Oriental Yeast Industries, Tokyo. S-9 Mix containing 30% S-9 by volume was used.

E. coli B/r WP2 *trpE65*³⁾ and *S. typhimurium* TA 98 *hisD3052*⁴⁾ were used. Cultures were grown in VYB medium (25 g of veal infusion broth and 5 g of yeast extract/l). Minimum synthetic medium (a modified Vogel-Bonner's) was prepared by dissolving 1 g of (NH₄)₂SO₄, 10 g of KH₂PO₄, 0.1 g of MgSO₄·7H₂O, and 0.5 g of trisodium citrate·2H₂O in distilled water; the solution was neutralized by KOH, then filled up to 1,000 ml. The semienriched agar medium (MB agar) for reverse mutation assays in *E. coli* contained per liter of above mixture, 4 g of glucose, 80 mg of nutrient broth (Difco), and 15 g of agar. A similar semienriched medium (MBB agar) for reverse mutation assays in *S. typhimurium* contained per liter of minimum synthetic medium, 4 g of glucose, 130 mg of nutrient broth (Oxoid), 100 ng of biotin, and 15 g of agar. Soft agar contained 0.6% NaCl and 0.6% agar.

E. coli cells grown for 8 hr in VYB medium at 37°C were

washed twice with 1/15 M phosphate buffer (pH 7.0), resuspended in the same buffer, and kept at 0°C. The source of UV irradiation was a 15 W germicidal lamp. For irradiation, 4 ml of the cell suspension in a 60 mm petri dish was exposed to UV (9.9 J/m²) at room temperature.

S. typhimurium cells grown for 8 hr in VYB medium at 37°C were washed twice with 1/15 M phosphate buffer (pH 7.0), and resuspended in the same buffer. The suspension was supplemented with AF2 stock solution to obtain the appropriate concentration (0.9 µg/ml) and kept at 37°C for 30 min. After the treatment, cells were washed twice with phosphate buffer and then resuspended in the phosphate buffer.

Linoleic acid was simply stirred at 37°C in a 1-l beaker. No catalyst was added during oxidation. The peroxide value (POV) was assayed as described by Lea⁵⁾ with a slight modification. Autoxidized linoleic acid (POV = 1110 meq/kg) was used for the experiments. The autoxidized linoleic acid dissolved in ethanol, 50 µl of mutated cell suspension, various amounts of S-9 Mix (0~800 µl), and 3 ml of molten soft agar were well mixed and poured onto an MB or MBB plate. The revertants were counted after incubation for two (*E. coli*) or three days (*S. typhimurium*) at 37°C.

The effects of S-9 Mix on antimutagenic activity on UV induced mutagenesis of *E. coli* B/r WP2 are shown in Table I. As reported previously,¹⁾ 5 mg of autoxidized linoleic acid reduced the number of mutant colonies (Trp⁺). In the presence of an antimutagenic dose of autoxidized linoleic acid (5 mg/plate), the number of mutant colonies was increased 3-fold (105→355) by the addition of S-9 Mix. If the increment depends on the production of an ultimate mutagen from autoxidized linoleic acid and S-9 Mix, the mutagen ought to increase Trp⁺ revertants in non-irradiated cells. As shown in Table I, however, such an effect was not seen at all in UV nonirradiated cells. On the other hand, I previously reported that autoxidized linoleic acid is lethal to genetic repair-deficient strains of *E. coli* without any addition of S-

TABLE I. EFFECTS OF S-9 MIX ON ANTIMUTAGENIC ACTIVITY OF AUTOXIDIZED LINOLEIC ACID (AOLA) IN UV INDUCED MUTAGENESIS OF *E. coli* B/r WP2

Treatment	Trp ⁺ revertants/plate	
	+ UV	- UV
AOLA 0 mg	1,153	3
AOLA 5 mg	105	7
AOLA 5 mg + S-9 Mix 200 µl	121	2
AOLA 5 mg + S-9 Mix 400 µl	195	6
AOLA 5 mg + S-9 Mix 600 µl	263	6
AOLA 5 mg + S-9 Mix 800 µl	355	7

Peroxide value of AOLA was 1110 meq/kg.

9 Mix.¹⁾ It is likely that autoxidized linoleic acid itself causes DNA damage without metabolic activation by S-9 Mix. These results suggest decomposition of antimutagenic activity rather than the formation of an ultimate mutagen. The results obtained above were confined to UV-induced mutagenesis in *E. coli*. To estimate these phenomena in other systems, I did the same experiments using AF2 induced mutagenesis in *S. typhimurium* TA 98. As shown in Table II, similar results were found in TA 98. In the presence of an antimutagenic dose of autoxidized linoleic acid (40 µg/plate) the number of mutant colonies was increased 6-fold (28→172) by the addition of S-9 Mix, but S-9 Mix could not increased the number of mutant colonies in AF2 non-treated cells.

S-9 Mix seems to decrease the antimutagenic effects of autoxidized linoleic acid on UV induced mutagenesis in *E. coli* and AF2 induced mutagenesis in *S. typhimurium*. When we consider the mutagenic effects of autoxidized fatty acids, therefore, the action of S-9 Mix will now be discussed not only from the aspect of metabolic activation but also by the aspect of decomposition of antimutagenic activity.

REFERENCES

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TABLE II. EFFECTS OF S-9 MIX ON ANTIMUTAGENIC ACTIVITY OF AUTOXIDIZED LINOLEIC ACID (AOLA) IN AF2 INDUCED MUTAGENESIS OF *S. typhimurium* TA98

Treatment	His ⁺ revertants/plate	
	+ AF2	- AF2
AOLA 0 µg	312	17
AOLA 40 µg	28	14
AOLA 40 µg + S-9 Mix 50 µl	28	9
AOLA 40 µg + S-9 Mix 100 µl	75	7
AOLA 40 µg + S-9 Mix 150 µl	127	14
AOLA 40 µg + S-9 Mix 200 µl	172	19

Peroxide value of AOLA was 1110 meq/kg.

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