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METHYL IODIDE, A POTENT INDUCER OF THE ADAPTIVE RESPONSE WITHOUT APPRECIABLE MUTAGENICITY IN E. coli

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SUMMARY Methyl iodide (MeI), a very weak mutagen, induced the adaptive response in E. coli to a similar extent to those induced by potently mutagenic methylating agents. MeI potentiated the mutagenicity of a methylating mutagen, N-methyl-N-nitrosourea, by its co-treatment. These results might give indication that MeI directly methylates O^6-methylguanine-DNA methyltransferase resulting in induction of the adaptive response and depletion of the repair capacity of enzyme.

It is likely that the genotoxic potency of methylating agents such as N-methyl-N-nitrosourea (MNU) depends on both how readily they produce O^6-methylguanine residues in cellular DNA and how effectively the cell eliminates the methyl group from the modified residues with the help of O^6-methylguanine-DNA methyltransferase. It is well documented in bacterial systems that this repair enzyme is inducible, encoded by the ada gene (1-3). This gene is activated by an S-methyl derivative of the gene product itself, which is formed by the methyl-transfer to the enzyme from O^6-methylguanine residues once produced in DNA by mutagenic methylating agents (4,5). Some assay systems have been established to quantify the capacity of chemicals in inducing the ada gene activation, i.e., the induction of an adaptive response toward a subsequent challenge by alkylating

Abbreviations: **MeI**, methyl iodide; **MNU**, N-methyl-N-nitrosourea; **MNNG**, N-methyl-N'-nitro-N-nitrosoguanidine, **MMS**, methyl methanesulfonate; **DMS**, dimethyl sulfate.

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agents (6). As for the chemicals assayed so far, all the potently mutagenic methylating agents effectively induce the adaptive response. Thus, methylating agents which are capable of producing O^6-methylguanine residues in cellular DNA are potently mutagenic and endowed at the same time with potent capacities in inducing the adaptive response.

This communication describes that methyl iodid (MeI), a common methylating agent in chemical laboratories, is endowed with a potent capacity to induce the adaptive response in the cell, although it is not a potent mutagen (7,8), i.e., it might have only a low capacity to produce the mutagenic lesion of O^6-methylguanine residue in DNA. As a probable mechanism for the ada-gene activation by MeI, we propose that MeI may directly methylate O^6-methylguanine-DNA methyltransferase, leading to the *ada* gene activation, not via the mutagenic methylation of guanine residues in DNA.

EXPERIMENTAL

Assay for the adaptive response induction The induction of adaptive response was assayed by the method developed by Sekiguchi and his co-workers (6), i.e., analyzing ß-galactosidase induced in the tester strain carrying *alkA'-lacZ'* fused gene. The strain used for the analysis was *E. coli* CSH26 which was transformed with pMCP1000 plasmid carrying *alkA'-lacZ'* fused gene, which was a gift from Professor Sekiguchi. The cells were grown in an LB medium containing 30 µg/ml of ampicillin at 37°C for 12 h. This culture was inoculated to a 100-times volume of M9 supplemented with 1% casamino acids, 1% glucose and 30 µg/ml of ampicillin. This was incubated at 37°C for 2 h, and then diluted to a 5-times volume with M9 supplemented with 1% casamino acids and 1% glucose. To 0.9 ml of this diluted culture, 0.1 ml of the chemical solution dissolved in dimethyl sulfoxide was added. After incubation at 37°C for 2 h with shaking, optical density at 600 nm was measured and ß-galactosidase activity was quantified.

Assay for ß-galactosidase activity The assay was carried out by the method of Miller (9) with slight modifications. The volume was 1 ml, and the reaction was carried out at 28°C for 30 min. The absorbances at 420 and 550 nm were measured with Shimadzu UV 210A spectrometer. The unit of enzyme activity was calculated according to the equation of Miller (9).

Procedure for cell adaptation The overnight culture of *E. coli* WP2 cells was diluted to 100-fold with medium E (10) supplemented with 1.4% glucose and 20 µg/ml of tryptophan. The

cell suspension was incubated at 37°C for 4-5 h until the cell density reached 0.30-0.35 OD at 660 nm. To this cell culture, 1/100 volume of the adapting agent was added. For control, the same volume of solvent was added. After incubation at 37°C for 90 min, the cells were collected by centrifugation at 3500 rpm for 20 min. The cells were washed with 1/15 M phosphate buffer (pH 6.8) and suspended in an equal volume of the same buffer.

Assay for mutagenicity The assay was carried out as described in our previous paper with a slight modification (11). The reaction mixture consisted of 0.75 ml of the phosphate buffer, 0.2 ml of an adapted or unadapted E. coli cell suspension, and 0.05 ml of challenging agents dissolved in dimethyl sulfoxide. This mixture was incubated at 37°C for 30 min with shaking. The mutation frequency was calculated as $(M-M_0)/N$, where M and M_0 are the numbers of revertant colonies obtained from the test compound-containing reaction mixture and the control mixture, respectively, and N is the number of surviving colonies obtained from 1 ml of the test compound-containing reaction mixture.

RESULTS

Induction of the adaptive response in E. coli CSH26/pMCP1000 (alkA'-lacZ')

Induction of the adaptive response was quantified by analysis of ß-galactosidase released after treatment of the tester cells with MeI. The dose-response curve thereby obtained is shown in Fig. 1 together with those of some other mutagenic methylating

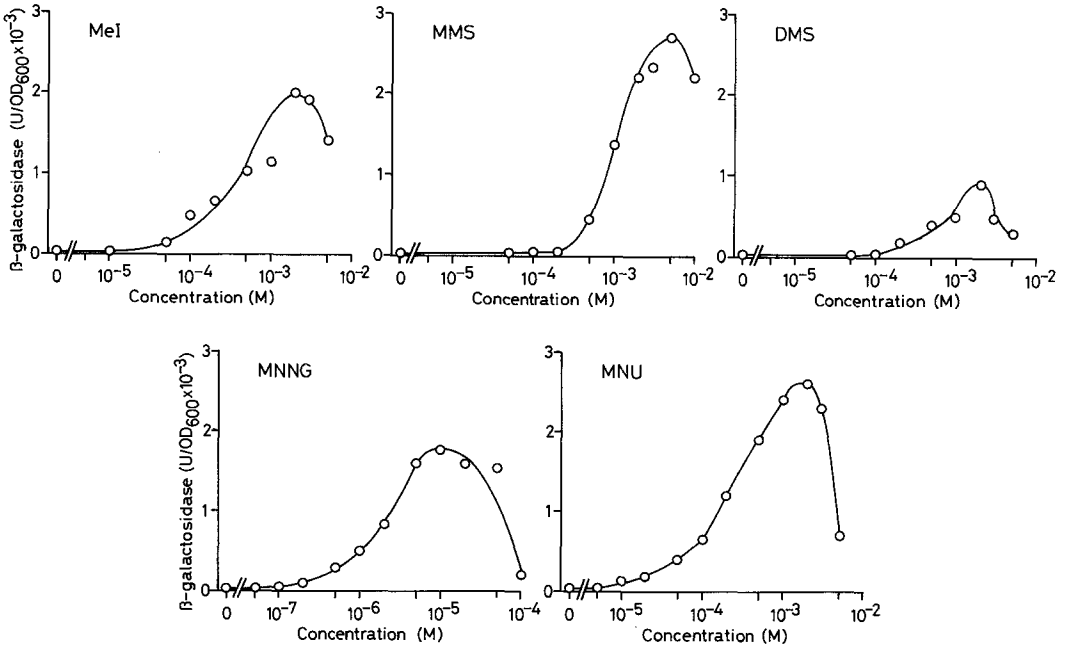

Figure 1. ß-Galactosidase activities induced by methyl iodide and some mutagenic methylating agents in the strain E. coli CSH26/pMCP1000 carrying alkA'-lacZ' gene

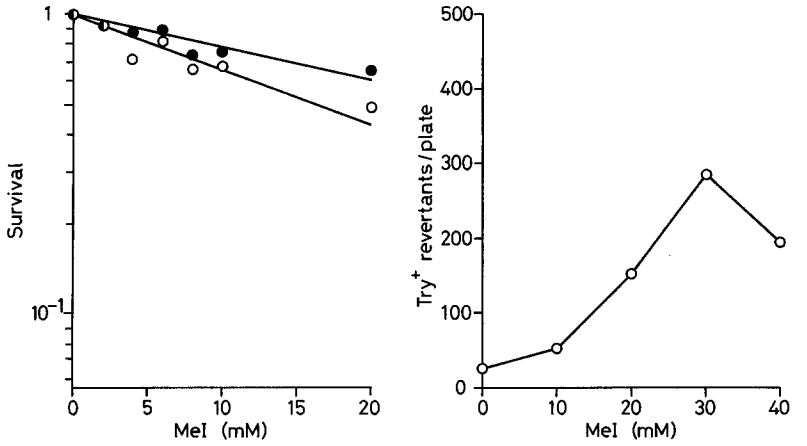

Figure 2. Killing and mutagenic effects of methyl iodide in E. coli WP2 cells
Left: survival fractions in (O) the unadapted cells and (●) in the adapted cells by pretreatment with N-methyl-N'-nitro-N-nitrosoguanidine, respectively Right: number of revertants in the unadaped cells

agents, MNNG, MNU, MMS, and DMS. The adaptive response induced by MeI was of a similar extent to those induced by all the potently mutagenic agents examined. Thus, MeI is a potent activator of the alkA gene.

Mutagenicity in E. coli WP2 strain

As previously reported, MeI showed no mutagenicity in S. typhimurium TA100 (7.8). In the present study, however, we demonstrated very weak mutagenicity of MeI in E. coli WP2 by a preincubation assay method, as shown in Fig. 2. It is worth noting that no revertants were induced in a dose range (up to ca. 1 mM) of MeI, where the adaptive response was potently induced. Then, its mutagenicity was tested in the same tester cells which were pretreated with a sublethal dose (3 μM) of MNNG, an inducer of the adaptive response (12). The adapted cells became resistant to lethal effect of MeI and any revertants were not thereby induced over the background level. Thus, MeI is capable of methylating guanine residues in DNA indeed, but its capacity

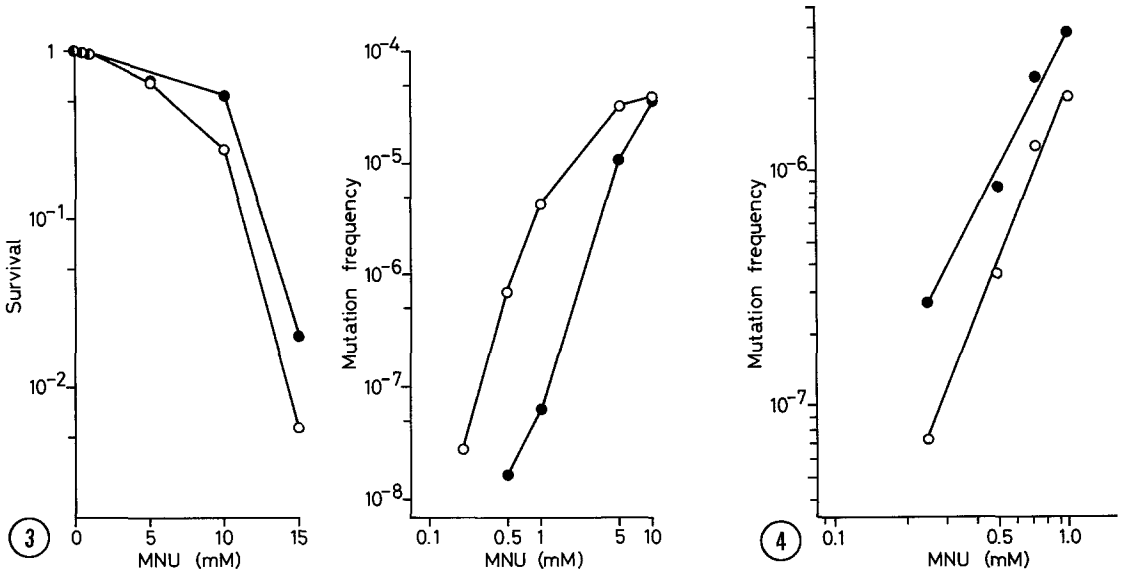

Figure 3. Killing and mutagenic effect of N-methyl-N-nitrosourea in E. coli WP2 cells adapted by pretreatment with methyl iodide (●) in the adapted; (O) in the unadapted

Figure 4. Mutagenicity of N-methyl-N-nitrosourea in E. coli WP2 cells in the presence (●) and absence (O) of methyl iodide in a non-growth medium

must be much lower than all the other methylating agents examined.

Effect of MeI-pretreatment on lethality and mutagenicity of MNU in E. coli WP2 strain

After E. coli WP2 cells were treated in a growth medium with a submutagenic dose (0.5 mM) of MeI, they were challenged by mutagenic doses of MNU. The MeI-pretreated cells were clearly more resistant to both MNU-induced lethality and mutagenicity than the untreated cells, as shown in Fig. 3. Thus, it is confirmed that MeI is an inducer of the adaptive response, as suggested by the colorimetric test for the alkA gene activation.

Simultaneous treatment of E. coli WP2 cells with MeI and MNU in a non-growth medium

Mutagenicity of MNU in E. coli WP2 was examined in the presence of a submutagenic dose (0.5 mM) of MeI. The

dose-response plot shown in Fig. 4 suggests that MeI inactivated the repair enzyme to result in potentiation of the mutagenicity of MNU.

CONCLUSION

Although MeI was found to induce mutation probably through production of O^6-methylguanine residues in cellular DNA, its mutagenic capacity was much lower than that expected from its high capacity to induce the adaptive response. The mutagenicity of MNU was potentiated by the co-treatment with MeI, suggesting that MeI inactivates a part of constitutive O^6-methylguanine-DNA methyltransferase probably through its direct methylation (13). These results seem to suggest that the induction of adaptive response by MeI might be mediated mainly through direct methylation of O^6-methylguanine-DNA methyltransferase, but not through methyl-transfer to the methyltransferase from the O^6-methylguanine once produced in cellular DNA. In conclusion, it can be said that MeI is a potent inducer of the adaptive response without an appreciable capacity to induce mutation and that the mechanism for activation of the *ada* gene might involve direct methylation of the methyltransferase, the *ada* gene product.

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