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E. coli Mutants sensitive to Alkylating agents and their Complementary Gene

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알킬화제 시약에 대해 민감한 *E. coli* 변종들과 그들의 상보적인 유전자에 대한 연구

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ABSTRACT: Mutants of *E. coli* which showed increased sensitivity to MMS(methyl methane sulfonate) were isolated by MNNG mutagenesis and characterized by enzymatic assay, survival of simple alkylating agents and host-cell reactivation. *E.coli* mutant, 5-62, which showed absolute deficiency in 3-methyladenine DNA glycosylase II activity and had low capability of reactivating MMS-treated phage charon 35 was very sensitive to MMS and MNNG. MMS^r gene which confered resistance to the lethal effects of MMS was cloned in 5-62 strain.

5-62 mutants carrying recombinant plasmid, pMRG 1, which acquired resistance to the lethal effects of MMS had normal sensitivity to MNNG. Resistance to MMS was somewhat increased after they were treated with 0.5 μg MNNG/m*l* for 2 hours at 37°C. Although recombinant plasmid, pMRG 1, did not complement alk A mutation in 5-62 and ada mutation in 1-27 mutant, mutants transformed with this plasmid showed more capability of reactivating MMS treated phage than mutants.

KEY WORDS □ MNNG mutagenesis, MMS-sensitive mutant, Host-Cell Reactivation, Recombinant plasmid pMRG 1.

Alkylating agents are electrophilic reagents which can combine with DNA and other cellular macromolecules. Different alkylating agents have different biological properties; for example, N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) produces a different pattern of mutaions and is more carcinogenic compared with methylmethanesulfonate(MMS) (Malling and de Serres, 1969). This difference in biological effect is a result of a specificity of chemical reaction, since each different alkylating agent gives a different but characteristic "mix" of reaction products, particulary of minor reaction products. The major reaction product of methylating agent with DNA is 7-methylguanine, and the properties which the alkylating agents have in common are likely to be due in some way to the formation of this substance. However, alkylation of all purines and pyrimidines can be demonstrated, and most of the organic bases react at several sites (Table 1). Although 7-methylguanine is the major product of methylating agents reacting with DNA, this adduct

is ignored by the bacterial and mammalian repair enzymes(Lawley and Orr, 1970) and is replicated without problem(Prakash and Strauss, 1970).

MNNG react with DNA to form a greater proportion of the chemically stable 6-methoxyguanine than does MMS.

Bacteria, as well as higher cells, have ability to repair several forms of DNA alkylation dammage. One of the two pathways that repair alkylated DNA is the action of 3-methyladenine DNA glycosylase I which removes one of the major alkylation products, 3-methyladenine from DNA. The other is inducible pathway that is turned on when *E. coli* cells are exposed to a low level of simple alkylating agents. This adaptive response is associated with two distinct repair enzymes, O^6-methylguanine methyltransferase and 3-methyladenine DNA glycosylase II. 6-Methoxyguanine is corrected by a transfer of the methyl group to a cysteine residue in the O^6-methylguanine methyltransferase(Olsson and Lindahl, 1980 ; Demple *et al.*, 1982), while N-alkylated purines such as 3-methyladenine(3-meA) and 3-methylguanine (3-meG), and O-alkylated pyrimidines such as O^2-methylcytosine and O^2-methylthymine are removed by 3-methyladenine DNA glycosylase II. To analyze the mechanisms of repair of alkylated DNA in *E. coli*, MMS-sensitive mutants were isolated and recombinant plasmid carrying mms^+ gene was constructed. Mutants carrying this recombinant plasmid were examined for biological effects of simple alkylating agents.

MATERIALS AND METHODS

Bacterial strains, bacteriophage and plasmids

E. coli LCB 850(CGSC 6408 ; F^-, hyd-1, leuB 6, thi-1, lacY 1, rpsL 175, tonA 21, lamda$^-$, supE 44) was used as a wild type strain. *E. coli* K 12 W 1485 was used as a source of chromosomal DNA. Phage charon 35 was used as a MMS-damaged phage. The vector plasmids pBR 322(Boliver, F. *et al.*, 1977) and pEMT 2(Baik. Y.J. *et al.*, 1986) were used as control vectors of pMRG 1. Plasmid pAAR 6 and pMK 4 were used as size markers.

Chemicals

MMS(Methylmethanesulfonate) was purchased from Aldrich. MNNG(N-methyl-N′-nitro-N-nitrosoguanidine) was obtained from sigma. Radioactive N-^{3}H-methyl-N-nitrosourea(2Ci/mmol) was purchased from Amersham. 7-methylguanine, O^6-methylguanine and 3-methyladenine were purchased from Cyclo chemicals. Restriction enzymes and T4 DNA ligase was obtained from Biolab. CIP(Calf-Intestinal phosphatase) was purchased from Pharmacia.

(I) Isolation of MMS-sensitive mutants

Exponentially growing cells of *E. coli* LCB 850 were treated with 100 μg of MNNG per m*l* at 37℃ for 15 min. in 50 mM tris(hydroxymethyl) aminomethane(Tris)-maleate buffer, pH 6.0(Adelberg. E.A. *et al.*, 1965). The cells were washed, resuspended in LB broth, and incubated overnight at 37℃. After appropriate dilution, bacteria were plated on LB-agar plates, and colonies formed were transferred to LB-agar plates containing 6.0 mM MMS by replica plating. After incubation overnight at 37℃, MMS-sensitive colonies were picked up from the master LB-plate and purified. All MMS-sensitive colonies were further tested for the resistance to UV-light at a dose of 25% survival of LCB 850. One of these strains, 5-62 was characterized by enzymatic assays, alkylating agent-sensitivity and host-cell reactivation.

(II) Characterization of MMS-sensitive mutant, 5-62

Adaptation: Cells were grown in M9CA medium to about mid-log phase at 37℃, and then MNNG was added to a final concentration of 0.5 μg/m*l* and incubated for further 60 min. at 37℃.

Determination of protein concentration: Protein concentration was determined by Coomassie Blue-binding protein assay(Spector,

T., 1978 ; Sedmak, J.J., 1977).

Sensitivity to MMS: a) Bacteria were cultured overnight at 37℃, and then this culture was appropriately diluted and plated on LB-agar plates with or without MMS. After incubation overnight at 37℃, the number of colonies formed was counted. b) 1% (118 mM) solution of MMS was prepared by dissolving MMS in M9S buffer(Yamamoto, Y. *et al.*, 1978) immediately before use. Bacteria were treated with 40 mM MMS in M9S buffer at 37℃. After an interval of 10 min., the cells were plated on LB-agar plates and then were incubated overnight at 37℃.

Sensitivity to MNNG: Bacteria were cultured overnight at 37℃, and then this culture was appropriately diluted and plated on M9CA(pH 6.0)-agar with or without MNNG. After incubation overnight at 37℃, the number of colonies formed was counted.

Assay of 3-methyladenine DNA glycosylase II: To prepare substrates, 1 mg of Calf-thymus DNA (2 mg/m*l* in 0.02 M ammediol buffer, pH 10.0) was reacted with 0.1 mCi ^{3}H-MNU and incubated 15 min. at 37℃. The alkylated DNA was recovered by spooling, washed in ethanol and ether, suspended in 5 m*l* of 0.05 M Tris-Cl(pH 7.6) and dialyzed against the same buffer(Cathcart, R. and Goldthwait, D.A., 1981 ; Lawley, P.D. *et al.*, 1973). This substrate had a specific activity of 2500-3000 cpm/n mol nucleotide.

Cell free extracts were prepared as described by Yamamoto *et al*(Yamamoto, Y. *et al.*, 1978). 0.1 M*l* of reaction mixture contained 0.07 M HEPES-KOH, pH 7.8, 1 mM EDTA, 5% glycerol, 1 mM 2-mercaptoethanol and H-MNU treated DNA (4-5 mg, 40,000-50,000 cpm). 3-methyladene was also included in 6 mM concentration when necessary. Reaction was started by addition of crude extract which contained 50-100 mg protein. After incubation at 37℃ for an appropriate time, the reaction was stopped by chilling to -20℃. Two volume of cold ethanol was added the whole mixture was centrifuged. Radioactivity of the supernatant was detected by liquid scintillation counting.

In chromatographic assay; The above precipitates were throughly suspended in 30 μl of 0.1 M HCl. DNA was hydrolyzed by incubation of this solution for 30 min at 70℃. Each hydrolysate, supplemented with carrier O^6-methylguanine, 7-methylguanine and 3-methyladenine, was applied to whatman 3 MM paper(or Cellulose thin layer) and chromatographed in isopropanol/conc. ammonia/water (7 : 1 : 2). Proper portions were cut out, extracted with 0.5 m*l* of 0.01 M HCl, and counted for radioactivity.

Host-cell reactivation; Phage charon 35 (3×10^6 plaque forming units(PFU) per m*l*) was suspended in LB(or M9S) medium containing various concentrations of MMS and incubated for 25 min. at 37℃. The suspension was diluted and 0.1 m*l* of the dilutions was mixed with 0.1 m*l* of plating bacteria (1.5×10^9 cells/m*l*). After incubation for 20 min. at 37℃, this mixture was added to 3.0 m*l* of LB-soft agar medium at 47℃. And then, the soft agar-bacterial suspension was poured onto a plate containing LB-hard agr. To obtain a firmer agar surface, the plates at this stage were placed at 4℃ for 1 hour. After incubation overnight (9-10 hr.) at 37℃, the number of plaques formed was counted.

(III) Cloning of mms$^+$ gene

The chromosomal DNA of *E. coli* K12 was purified by the modified Marmur's method(Marmur, J., 1961). Large scale purification of pBR 322 was performed by the modification of alkaline method(Birnboin, H. and Doly, J., 1979). Linear plasmids such as pBR 322 (4.3 kb), pAAR 6 (7.86 kb) and pMK 4 (5.6 kb) were used as molecular weight markers. The DNA restriction fragments were analyzed on 0.7% agarose gels and stained with ethidium bromide for visualization under UV-light. The chromosomal DNA and vector plasmid pBR 322 were digested with restriction enzyme, Pst I, Hind III and EcoR I. Restriction fragments of vector DNA were dephosphorylated with CIP(calf-intestimal phosphatase) at 37℃. The phosphatase was

removed by phenol/chloroform extraction. And then, the chromosomal DNA and vector DNA were ligated with two-fold excess of vectors(total DNA conc. 300 $\mu g/ml$) at 16℃ for 18 hours.

Transformations were performed by the calcium chloride procedure(Maniatis, T. *et al.*, 1982).

Screening of mms$^+$ gene; Recombinant plasmids were screened for their ability to confer resistance to lethal effects of MMS on MMS -sensitive mutant, 5-62. 5-62 strain was transformed with stocks of recombinant plasmids constructed in vitro by ligation of restriction endonuclease-cleaved chromosomal DNA and pBR 322. The transformed cultures were grown overnight in LB medium containing antibiotics(Ampicilline, 50 $\mu g/ml$; Tetracycline, 12.5 $\mu g/ml$). After an appropriate dilution, aliquots were plated on LB-agar containing antibiotics and MMS(2.4 mM).

(IV) Examination of the biological effects of gene in the mutant

Sensitivity to simple alkylating agents such as MMS and MNNG and activity of 3-methyladenine DNA glycosylase II were tested for mutant, 5-62 with or without the cloned gene. And change of sensitivity to MMS was examined for 5-62 carrying the cloned gene after the baterial cultures were treated with several doses of MNNG for various intervals at 37℃.

In order to study the function of mms$^+$ gene, which confered resistance to lethal effects of MMS, recombinant plasmid possessing this gene, pMRG 1 was introduced into mutant, 1-27, which showed deficiency in adaptive response. And then, sensitivity to MMS and MNNG was investigated with 1-27(pMRG 1) and 5-62(pMRG 1) in comparision with simple mutants and 1-27(pEMT 2). (Recombinant plasmid, pEMT 2, which complemented Ada mutation had been constructed in our lab.) Finally, host-cell reactivation using phage charon 35 was examined for 5-62(pMRG 1) and 1-27(pMRG 1).

RESULTS AND DISCUSSION

(I) Isolation and Characterization of MMS -sensitive mutants

LCB 850 was mutagenized with MNNG and MMS-sensitive mutants were isolated by replica-plating method. Two MMS-sensitive mutants with same MMS-sensitivity were isolated. These two mutants named 1-45 and 5-62 were resistant to UV as parental strain. Fig.1, 2, 4 showed sensitivity of these strains to MMS in comparision with other mutant, 1-27(Baik, Y.J. *et al.*, 1986). Fig.3 compared toxicity of MNNG to several strains. Unlike 1-27 mutant, 5-62 mutant was very sensitive to MMS. Plating efficiency on LB-agar containing 2.4 mM MMS was 0.98% for 5-62.

Host-cell reactivation; There is a possibility that MMS-sensitive mutant might be a permeability mutant that takes up MMS more efficiently than does the wild type strain. It was

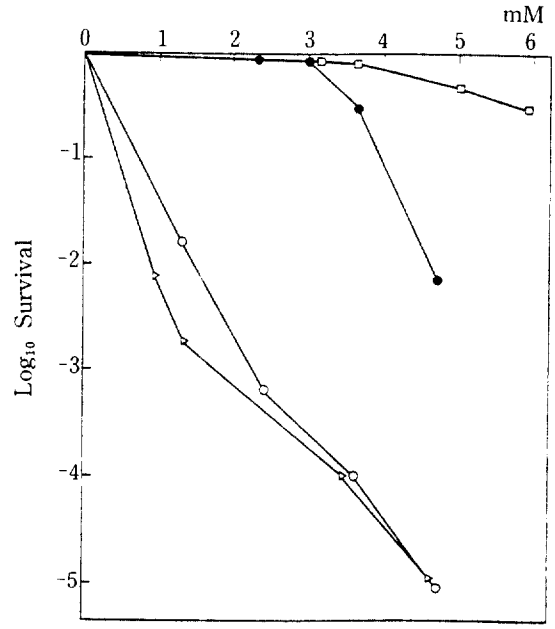

Fig.1. *Survival of E. coli strains on LB plates with MMS*

□ LCB 850 ○ 5-62(pBR 322)
△ 1-45 ● 5-62(pMRG 1)

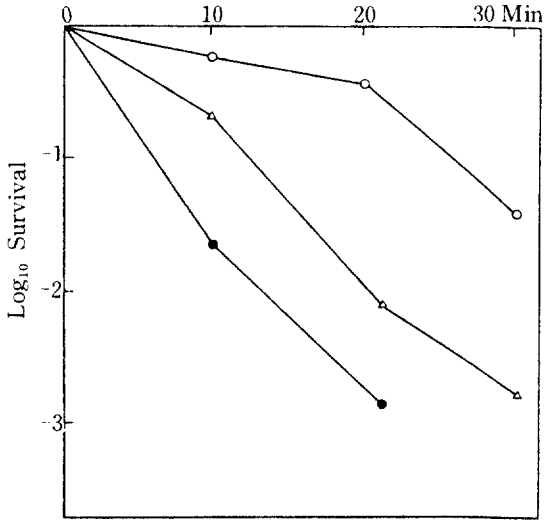

Fig.2. *Survival of E. coli strains pre-exposed to MMS on LB plates.*
○ LCB 850 ● 5-62
△ 5-62(pMRG 1)

examined whether phage pre-exposed to MMS can be reactivated by the mutant. In these experiments LCB 850, 5-62 and 5-62(pMRG 1) were used. As shown in Fig.5, 6, 5-62

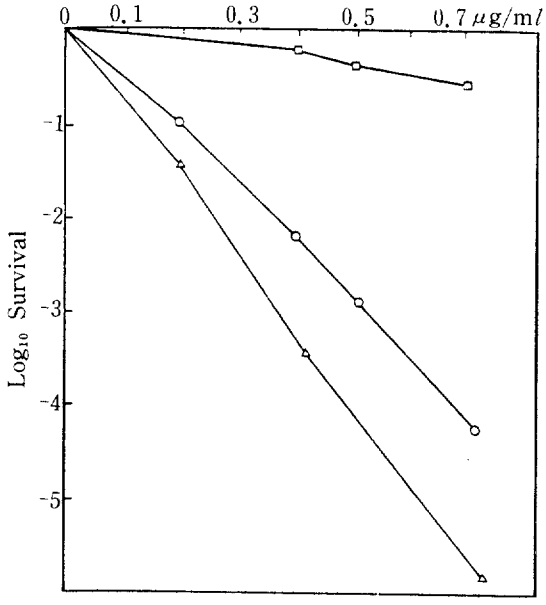

Fig.3. *Survival of E. coli strains on M9CA plates with MNNG.*
○ 5-62(pMRG 1) △ 5-62(pBR 322)
□ LCB 850(pBR 322)

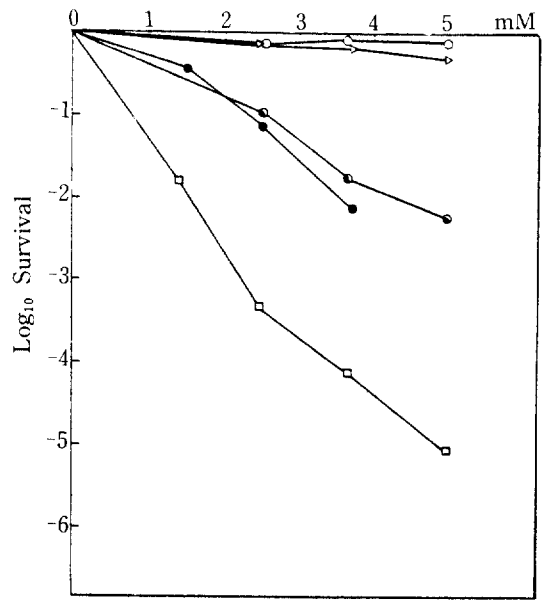

Fig.4. *Survival of E. coli strains on LB plates with MMS.*
○ 1-27(pMRG 1) ● 1-27(pEMT 2)
◑ 1-27(pBR 322) △ LCB 850(pBR 322)
□ 5-62(pBR 322)

mutant was less capable of reactivating MMS-treated phage charon 35. On the other hand, 1-27 mutant exhibited more capability for reactivating MMS-treated phage than 5-62 strain, but less than wild-type strain. Mutant , 1-27, which had normal constitutive level of 3-methyladenine DNA glycosylase II, was partially active in repairing DNA alkylation damage of phage charon 35 compared with 5-62 mutant which had absolute deficiency in 3-methyladenine DNA glycosylase II.

Enzyme activity of 5-62 mutant; The methylated bases in the substrate is shown in Fig.7. One of the major methylation products in DNA, 3-methyladenine is known to be actively and rapidly removed *in vivo*. The enzymes, 3-methyladenine DNA glycosylase I and II can be detected from unadapted cell(Thomas, L. *et al.*, 1982). Although only 5 to 10% of the total activity is due to glycosylase II in cells growing in normal medium, this activity can be induced to a 20-fold higher levels in cells exposed to low concentrations of alkylat-

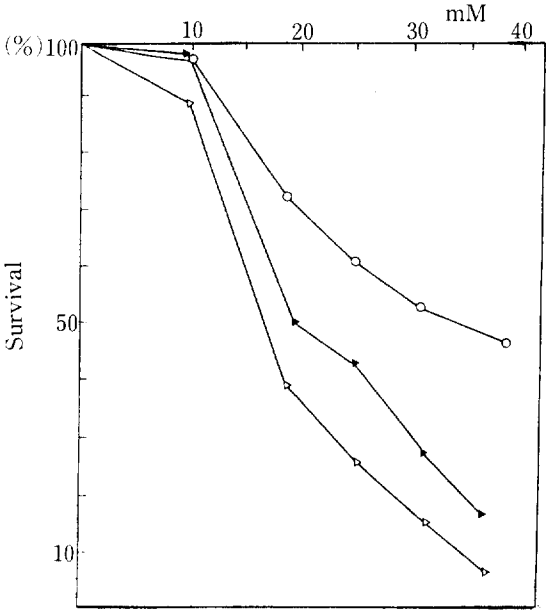

Fig.5. *Host-cell Reactivation: Survival of charon 35 exposed to MMS.*
○ LCB 850(pBR 322) ▲ 5-62(pMRG 1)
△ 5-62(pBR 322)

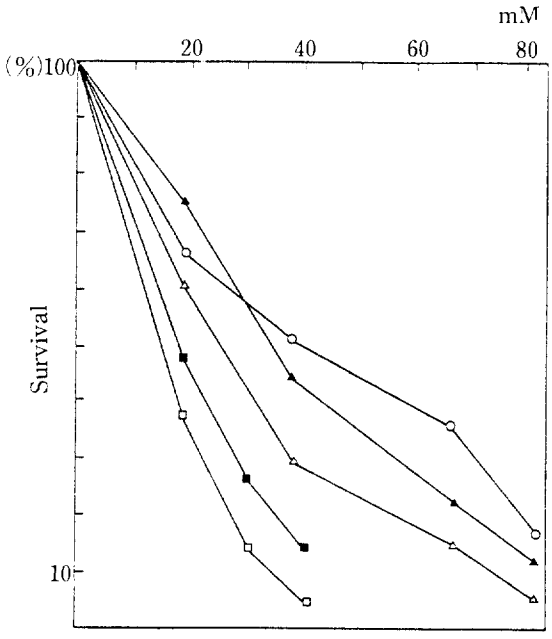

Fig.6. *Host-cell Reactivation Survival of charon 35 exposed to MMS.*
○ LCB 850(pBR 322) □ 5-62
△ 1-27 ■ 5-62(pMRG 1)
▲ 1-27(pMRG 1)

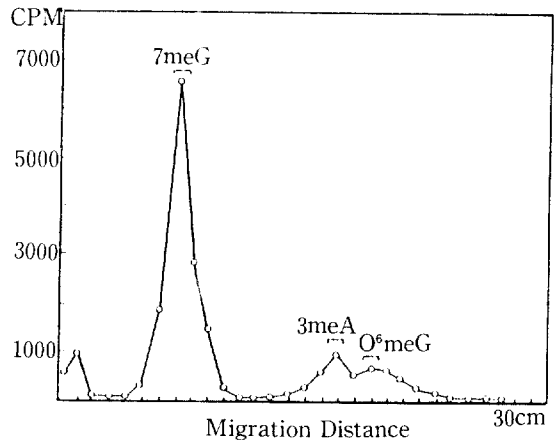

Fig.7. *The composition of ^{3}H-MNU treated DNA Hydrolysates was applied to whatman 3 MM paper and chromatographed in isopropanol/conc.Ammonia/water(7 : 1 : 2). Proper portions were cut out, extract with 1 ml 0.01 M HCl and counted for radioactivity.*

ing agents(Sedwick, B., *et al.*, 1980; Yamamoto, Y. *et al.*, 1978). Therefore in adapted cells this enzyme was responsible for 50-70% of the total activity. 3-methyladenine DNA glycosylase II has properties different from those of DNA glycosylase I; it is relatively heat resistant, and has broader substrate

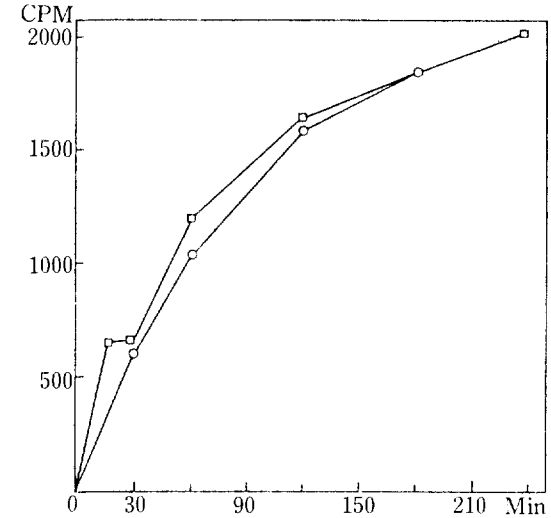

Fig.8. *Activity of 3-methyladenine DNA glycosylase II.*
□ 5-62 ○ No-extract(control)

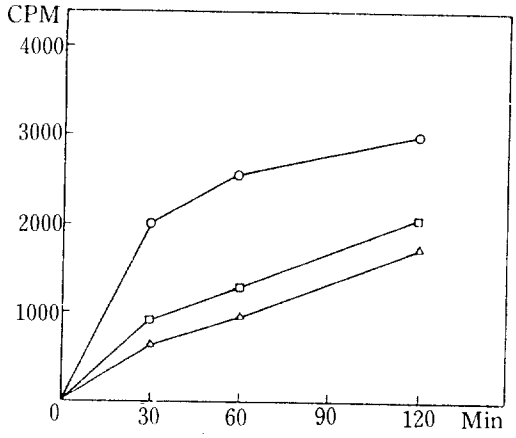

Fig.9. *Inhibition test: Activity of 3-methyladenine DNA glycosylase II with 3-methyladenine.*
○ 0 mM □ 3 mM △ 6 mM

specificity. The activity of glycosylase II was inhibited by 6 mM of 3-methyladenine (Fig. 9). In this condition, DNA glycosylase II activity of several strains was assayed(Fig.10, 8). Another mutant, 1-45, was also deficient in 3-methyladenine DNA glycosylase II. It

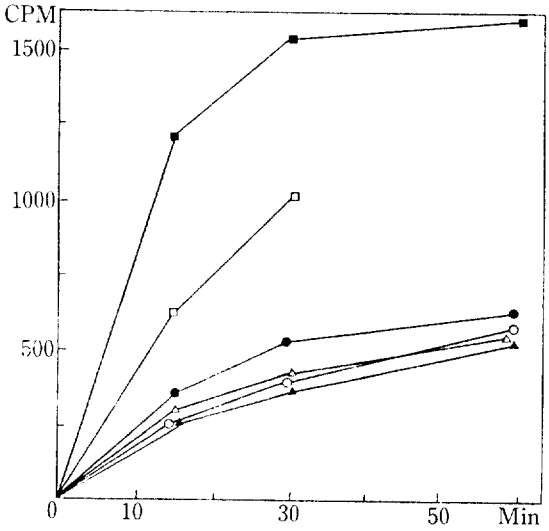

Fig.10. *Activity of 3-methyladenine DNA glycosylase II.*
□ LCB 850 : Non-induced
■ LCB 850 : Induced
○ 5-62(pMRG 1) : Non-induced
● 5-62(pMRG 1) : Induced
△ 5-62 : Non-induced
▲ 5-62 : Induced

was examined whether 5-62 mutant had defect in ada gene by complementation test with the cloned ada gene(recombinant plasmid pEMT 2). 5-62 Mutant carrying recombinant plasmid pEMT 2 had the same sensitivity to MNNG as bare 5-62 mutant.

(II) Cloning of mms$^+$ gene(Construction of recombinant plasmid pMRG 1)

Chromosomal DNA from *E. coli* K12 wild -type strain was partially digested with restriction enzyme, Pst 1. Digested chromosomal DNA and CIP-treated pBR 322 after digestion were mixed in 2 to 1 ratio and ligated at a final conc. of 300 μg/m*l*. 5-62 strain was transformed with these recombinant plasmids. This transformed culture was grown overnight in LB containing tetracycline(12.5 μg/m*l*). After an appropriate dilution, overnight cultures were plated on LB-agar containing MMS (2.4 mM) and tetracycline(12.5 μg/m*l*). Under this condition, the 5-62 mutant transformed with pBR 322 vector alone showed no colony on this plate. When 5-62 mutant with recombinant plasmid were spread on it, 10-20 colonies appeared on the plate. All colonies which survived this screening condition had the same 8.4 kb plasmid, pMRG 1. The 5-62 mutant retransformed with pMRG 1 all survived at 2.4 mM MMS plate, while the 5-62 mutant retransformed with pBR 322 did not survive at all. Electrophoresis of Pst 1, Hind III, EcoR I and BamH I digested pMRG 1 was run on 0.7% agarose gel(Fig.11, 12).

(III) Examination of the biological effects of pMRG 1 on MMS-sensitive mutants

Sensitivity to MMS and MNNG was examined for 5-62(pMRG 1), 5-62(pBR 322) and LCB 850(pBR 322)(Fig.1, 2, 3). Mutant strains carrying pMRG 1 acquired resistance to the lethal effects of MMS but not MNNG. Activity of DNA glycosylase II was investigated for above strains. But no enzyme activity was detected in the extract of 5-62(pMRG 1)(Fig.10). It was inferred from the analysis of the restriction map of pMRG 1 that the cloned gene was different from other repair genes such as alk A(Nakabeppu, Y. and Se-

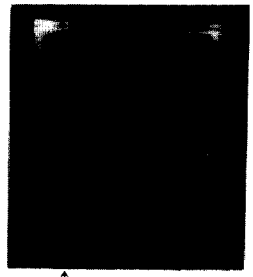

Fig.11. *Agarose gel electrophoresis of various plasmid DNA.*

Lane 1 : Intact pMRG 1
Lane 2 : pMRG 1 digested with EcoR I
Lane 3 : pMRG 1 digested with Hind III
Lane 4 : Linear plasmids; pBR 322(4.3 kb), pAAR 6(7.86 kb), pMK 4(5.6 kb)
Lane 5 : pMRG 1 digested with Pst 1
Lane 6 : pMRG 1 digested with Hind III and Pst I
Lane 7 : pMRG 1 digested with BamH I and Pst I
Lane 8 : pMRG 1 digested with BamH I

kiguchi, 1985), ada(Teo, I., *et al.*, 1984), alk B(Kataoka. *et al.*, 1985) and tag 1(Clarke, N. D., *et al.*, 1984).

On the other hand, 1-27 mutant, when transformed with recombinant plasmid pMRG 1, also showed resistance to the toxicity of

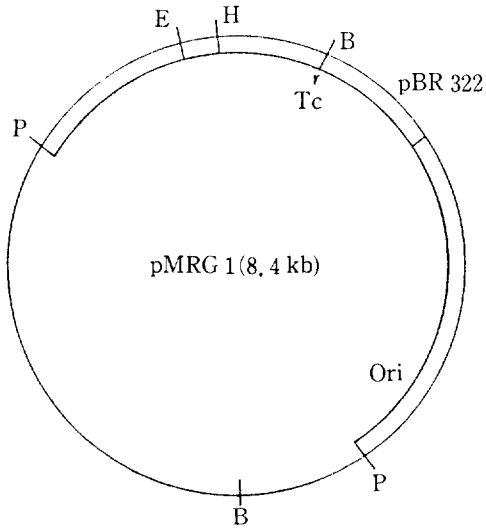

Fig.12. *The restriction map of pMRG 1.*
P: Pst 1 site H: Hind III site
E: EcoR I site B: BamH I site

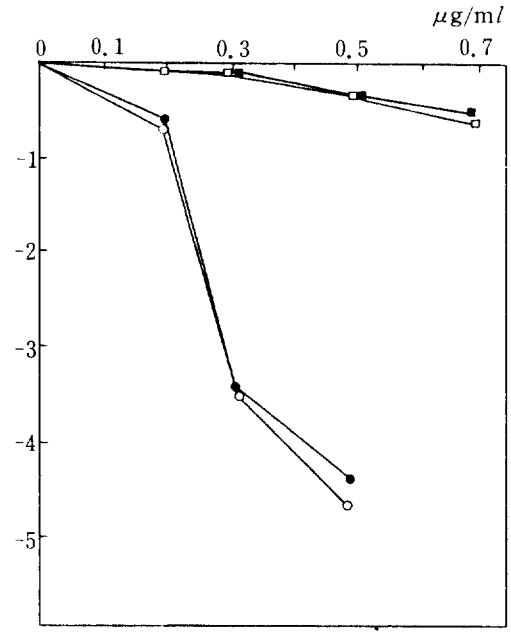

Fig.13. *Survival of E. coli strains on M9CA plates with MNNG.*

○ 1-27(pBR 322) ■ 1-27(pEMT 2)
● 1-27(pMRG 1) □ LCB 850(pBR 322)

MMS(Fig.4) but not MNNG(Fig.13).

The culture of 5-62(pMRG 1) treated with several induction doses of MNNG for various intervals at 37℃ showed changes in sensitivity to MMS. In this induction test, resistance to the lethal effects of MMS was somewhat increased when the induction dose of MNNG was 0.5 μg/ml for 2 hours at 37℃(Fig.14).

In the experiment of host-cell reactivation, 5-62(pMRG 1) was more capable of reactivating the MMS-treated phage charon 35 than 5-62(pBR 322) mutant(Fig.5). Failure of full increase in survival of phage might be explained by considering that 5-62(pMRG 1) was still deficient in 3-methyladenine DNA glycosylase II which repaired 3-methyladenine, 3-methylguanine and O^2-methylpyrimidines. And the 1-27(pMRG 1) had also somewhat increased capability of reactivation of MMS-treated phage(Fig.6). It was inferred from these experimental results that the cloned gene(MMS$^+$ gene) might be related with some repairing action in *E. coli*.

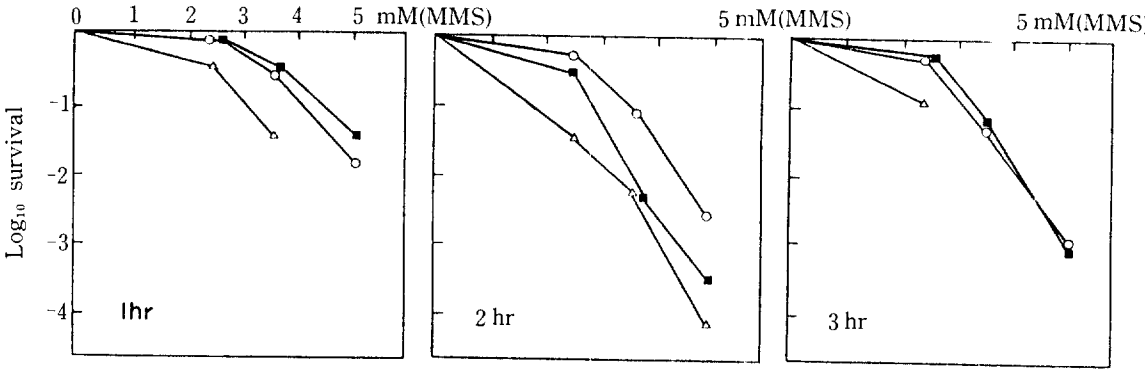

Fig.14. *Induction test; changes in MMS-sensitivity of 5-62(pMRG 1) pre-exposed to MNNG*
■ 0 μg/ml MNNG ○ 0.5 μg/ml MNNG △ 4 μg/ml MNNG

As mentioned before, MMS and MNNG have the different mechanisms of reacting with DNA and then they have different reaction sites with DNA(Table 1). Each methylated base(Fig.15) is repaired by two distinct repair enzymes such as O^6-methylguanine methyltransferase and 3-methyladenine DNA glycosylase II. McCarthy showed that O^2-methylcytosine and O^2-methylthymine were removed by DNA glycosylase II, and showed that O^4-methylthymine was repaired by methyltransferase, previously known to correct O^6-methylguanine by transfer of methyl group to one of its own cysteine residues(McCarthy, T.V., *et al.*, 1984).

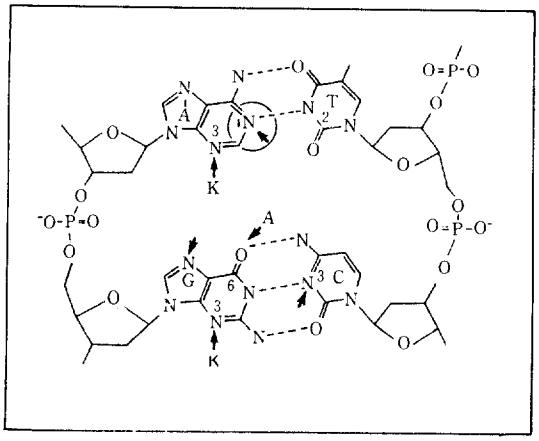

Fig.15. *Section of DNA reported sites of reaction with MMS(methylmethanesulfonate).*
A: ada gene product; reaction site of O^6-methylguanine methyltransferase
K: alk A gene product; reaction sites of 3-methyladenine DNA glycosylase II.

As shown in Fig.15, all the methylated bases, except 1-methyladenine, 3-methylcytosine and 7-methylguanine, are repaired from the DNA after treatment with MMS. As mentioned before, although 7-methylguanine is the major product(Table 1) of methylating agents reacting with DNA, this adduct is ignored by the bacterial repair enzymes(Lawley and Orr, 1970) and is replicated without problem (Prakash and Strauss, 1970). But, the methyl

Table 1. *Reported sites of attack by electrophilic reagents on DNA*

Compounds	Reaction sites				Reaction Type	Reference
	Adenine	Cytosine	Guanine	Thymine		
MNU	N-1 (1.4) N-3 (11.2) N-7 (2.5)	N-3 (1.6)	N-3 (1.1) N-7 (75.7) O-6 (7.3)	N-3 (0.3) O-4 (0.1)	SN1	a
MMS	N-1 (5) N-3 (9)	N-3 (1)	N-3 (0.68) N-7 (86) O-6 (0.34)		SN2	b
MNNG	N-1 (1) N-3 (12)	N-3 (2)	N-3 (2) N-7 (67) O-6 (7)		SN1	c

a: Lawley, P. and Shah, S. (1973) Chem. Biol. Interact. 7 : 115-120.
b: Lawley, P. and Shah, S. (1972) Chem. Biol. Interact. 5 : 286-288
c: Lawley, P. and Thatcher, C. (1970) Biochem. J. 116 : 693-707
* Values in parentheses give approximate amounts of reaction products. Lack of entry means only that the reference given does not record that product.

group bound to 1-methyladenine and 3-methylcytosine prevents these two bases from forming hydrogen bonds in DNA strands.

Therefore, it is more reasonable to think that enzymatic rapair of these two methylated bases are not found yet, than to think that there is no such activity.

It will be investigated further whether the cloned gene codes a protein which repairs these two methylated bases.

적 요

E. coli 의 한 변종인 LCB850 strain을 MNNG로 처리하여 MMS에 대해 증가된 민감성을 갖는 변종들을 분리하고, 이들에 대해 효소 활동도, 간단한 알킬화제 시약에 대한 민감성을 조사하고, bacteriophage을 이용한 숙주세포 재활성도 능력 평가 실험을 실시하여 이들을 특정지었다.

E. coli 의 변종인 5-62는 3-methyladenine DNA glycosylase II의 효소 활동도가 전혀 없었으며, 알킬화제 시약인 MNNG와 MMS에 대한 매우 증가된 민감성을 보였다. 또한 이 변종 5-62는 MMS가 처리된 phage charon 35을 숙주내에서 재활성화 시키는 능력이 현저히 부족하였다. 변종 5-62에서 MMS에 대해 증가된 저항성을 주는 MMS^+ gene을 cloning 하였다. 재조합 plasmid인 pMRG1은 변종 5-62에서 MMS에 대한 민감도를 감소시켰으나 MNNG에 대한 민감도는 변화시키지 못했다. 이 plasmid를 포함한 변종 5-62는 0.5 $\mu g/ml$의 MNNG를 37℃에서 2 시간 처리 하였을때 MMS의 저항성을 보다 촉진시켰다. 재조합 plasmid인 pMRG1이 alk A 변이와 ada 변이를 회복시키지 못했으나, MMS가 처리된 파지를 재활성화 시키는 능력은 이 plasmid가 없는 변종보다 증가시켰다.

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