Relation of the Repair of Damage Induced by a Monofunctional Alkylating Agent to the Repair of Damage Induced by Ultraviolet light in *Bacillus subtilis*[1]

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The monofunctional alkylating agent, methyl methanesulfonate(MMS) inactivates transforming DNA by a chain of processes which culminate in the insertion of single strand breaks into DNA(Strauss and Wahl, 1964; Wahl, 1965). MMS-induced damage can be repaired(Strauss, 1963, Reiter and Strauss, *in press*). Recently it was found (*loc. cit.*) that a mutant of *B. subtilis* sensitive to ultraviolet radiation(UV) was not sensitive to MMS. A simple way of accounting for this lack of MMS sensitivity in the *uvr*⁻(UV-sensitive) strain was to suppose that repair of biological damage, including UV damage, occurred by a sequence of reactions(Setlow and Carrier, 1964; Boyce and Howard-Flanders, 1964), and that a block in one of the earlier reactions did not inactivate the later steps. Repair of MMS damage might require only these later steps. In this paper we report on the properties of a newly isolated MMS-sensitive mutant which confirm some of the predictions of this hypothesis.

Materials and Methods

Transformation was performed according to the method of Anagnostopoulos and Spizizen(1961). NBS and CHT50 media were prepared as described by Bott and Strauss (1965). Phage SP01 was prepared and assayed as described by Okubo, Strauss and

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Stodolsky(1964). Methyl methanesulfonate(MMS) was purchased from Eastman Organic Chemicals and was vacuum redistilled before use. A 0.05M solution was prepared by dissolving 0.085 ml of MMS in 20 ml of solution immediately before use. Bacteria and free phage were irradiated with UV on a rotary shaker in open containers 45 cm from a 15 watt General Electric germicidal lamp giving a measured dose of about 20 ergs/ mm^2/sec at this distance.

An MMS sensitive mutant of B. subtilis was isolated by a modification of the method of Howard-Flanders and Theriot(1962). Strain JB1-49 (ind$^-$his$^-$cys$^-$uvr$^+$) was treated with N-methyl-N-nitro-N-nitroso-guanidine by the method of Adelberg, Mandel and Chen(1965). A culture was treated with 100 μg/ml to give 70 per cent survival. The cells were diluted 1:10, incubated 6 hours in CHT50, plated for viability and stored overnight in the refrigerator. Between 250 and 500 treated organisms were then plated on NBS and after 4 hr at 37 C a suspension of SP01 phage, previously incubated for 50 min with 0.025M MMS($N/N_o = 10^{-2}$), was gently poured over the bacteria. Between 10^6 to 10^7 infectious centers were added to each plate. We observed 599 colonies on 40 plates from the 15,000 treated bacteria plated. Of the 72 picked for study, 22 were phage resistant, 5 were of intermediate sensitivity and one, JB1-49 (59) was very sensitive to MMS. This strain will be designated mms$^-$. A uvr$^-$ strain of the proper genetic background for these experiments, JB1-49(23), was prepared by isolation of an ind$^+$uvr$^-$ derivative of JB1-49 by transformation using uvr$^-$ DNA as donor.

Results

The response of mms$^-$, uvr$^-$ and mms$^+$uvr$^+$ strains to MMS and to UV is shown in Fig. 1 and Fig. 2. The survival of MMS and UV treated SP01 assayed on the three strains is shown in Fig. 3 and Fig. 4. The differential response to these agents is emphasized by plotting the ratio: survival of phage on uvr$^-$ or mms$^-$ divided by survival of phage on uvr$^+$mms$^+$ as a function of survival of phage on uvr$^+$mms$^+$. The mms$^-$ strain was, of course, selected to be deficient in its ability to reactivate phage treated with

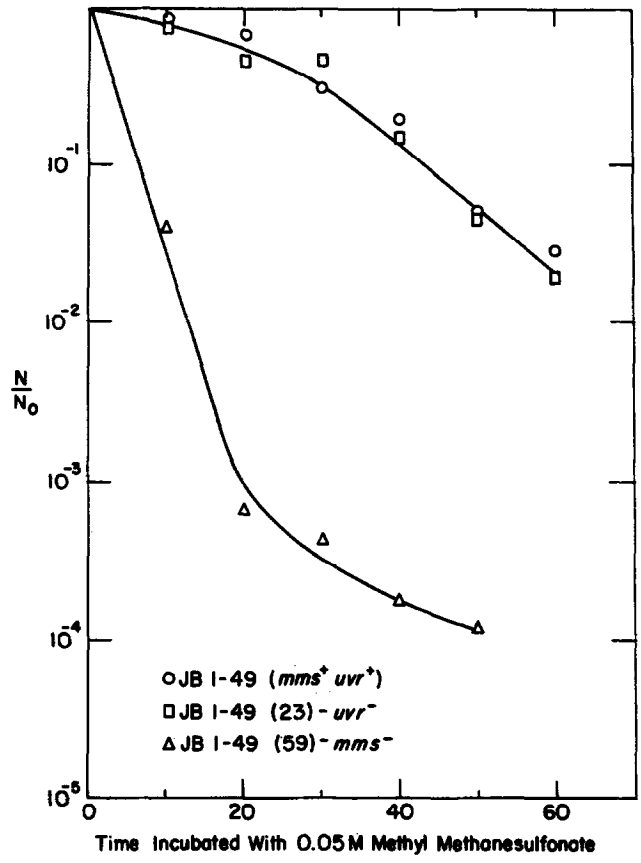

Fig. 1. Sensitivity of B. subtilis to MMS. An overnight culture was diluted into NBS, incubated 5 hr, diluted 1:10 with salts solution (Anagnostopoulos and Spizizen, 1961) and treated with MMS. Plating was on CHT50 medium solidified with 1.5% agar.

MMS. In contrast to the uvr⁻ strain previously reported (Reiter and Strauss, in press) to be no more sensitive to MMS than the wild-type, the mms⁻ strain was found to be sensitive to UV at higher doses.

The uvr⁻ strain acted as a recipient in transformation with the same efficiency as the uvr⁺. The mms⁻ strain was poorly transformable. At a DNA concentration of 26 μg/ml the best transforming frequency obtained at the indole locus with the mms⁻ strain as a recipient was 2.5×10^{-6} whereas the parental mms⁺ transformed at a

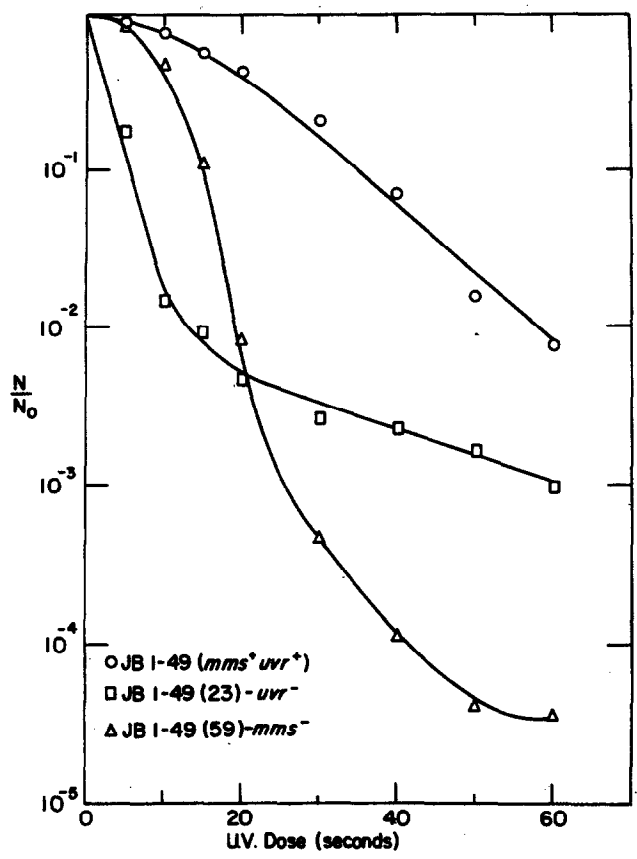

Fig. 2. Sensitivity of B. subtilis to UV. An overnight culture was diluted 1:10 into CHT50, incubated 5 hr at 37 C and then diluted 1:10 in salts solution for irradiation.

frequency of 1.1×10^{-3}. When SP01 phage DNA was used for transformation, phage were produced at a frequency of 6.2×10^{-4} per viable mms$^+$ cell, but no more than 2.5×10^{-8} of the mms$^-$ cells produced phage even though the same protocol was used to produce competent bacteria.

The inability of mms$^-$ organisms to repair MMS-induced damage was demonstrated by the following experiment: the ind$^+$ marker was introduced into mms$^-$ and mms$^+$ strains by transformation. Overnight cultures of these ind$^+$ strains were then prepared, harvested,

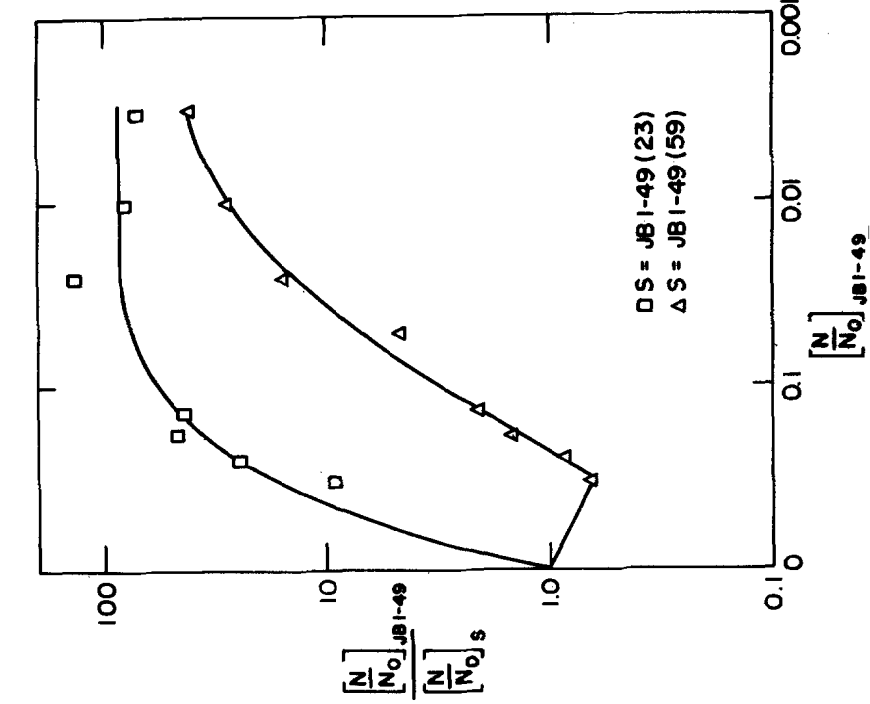

Fig. 4. Differential sensitivity of the host cell reactivation mechanism for UV treated phage SP01 in three strains of B. subtilis. Phage were irradiated for 0, 5, 10, 15, 20, 30, 40, 50 and 60 sec.

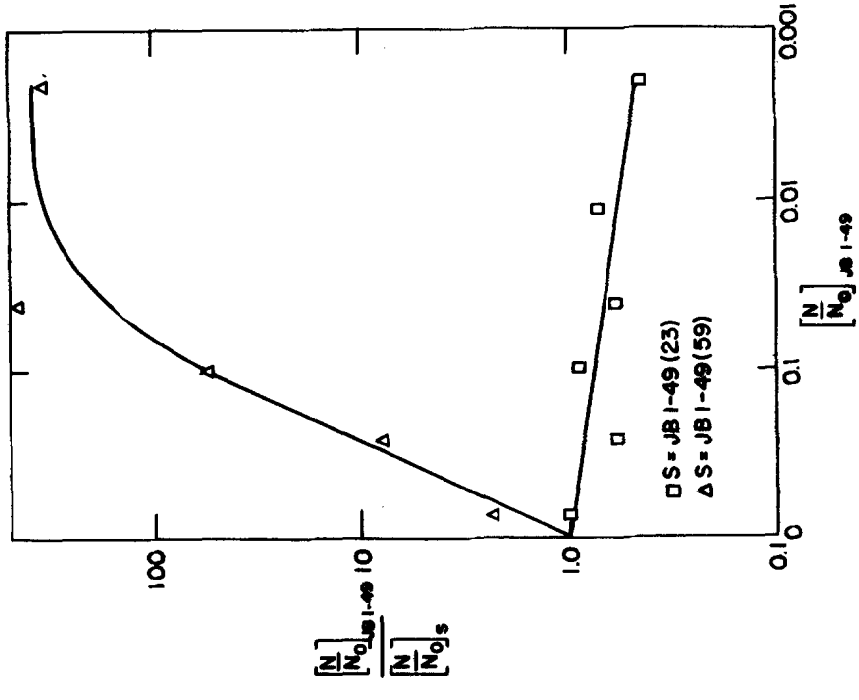

Fig. 3. Differential sensitivity of the host cell reactivation mechanism for MMS treated phage SP01 in three strains of B. subtilis. Phage were treated with 0.05M MMS for 0, 10, 20, 30, 40, 50 and 60 min.

washed and treated with 0.05M MMS for 30 min at 37 C. Cell lysates were prepared by successive treatment with lysozyme, pronase and Duponol (Reiter and Strauss, in press) either immediately or after 60 min incubation in CHT50. Little net DNA synthesis occurs under these conditions (Strauss, 1963). MMS treated mms^+ cells show a doubling in extractable transforming activity as a result of incubation in contrast to mms^- cells (Table 1). The initial amount of marker inactivation was similar in both strains indicating that the difference in survival was due to events subsequent to the MMS treatment.

Table 1. Recovery of mms^+ and mms^- strains from treatment with MMS

Strain	time incubated in CHT50(min)	other addition to CHT50	extractable ind^+ activity on an ind^-uvr^- recipient*
ind^+mms^+	0	-	0.15
	60	none	0.27, 0.34
	60	10^{-2}M NaCN	0.20
ind^+mms^-	0	-	0.12
	60	none	0.071, 0.051
	60	10^{-2}M NaCN	0.023

* relative to the extractable transforming activity from non-treated cultures incubated 60 min in CHT50. Transformation assay values were taken from the linear region. Surviving fractions after MMS: $mms^+ = 4.6 \times 10^{-3}$, $mms^- = 4 \times 10^{-7}$

Discussion

The repair of UV-induced damage in DNA probably occurs by a sequence of at least three major steps: a) excision of the damaged DNA strand containing the thymine dimers formed by UV along with an adjacent segment of the chain, b) repair replication of the excised region using the non-damaged strand as template and c) final connection of the repaired region to the end of the remaining "old" strand(Boyce and Howard-Flanders, 1964; Setlow and Carrier, 1964). This repair mechanism also operates in the repair of DNA

damaged by the bifunctional alkylating agents sulfur mustard(Lawley and Brookes, 1965) or nitrogen mustard(Hanawalt and Haynes, 1965; Kohn, Steigbigel and Spears, 1965). Damage induced by an agent such as MMS which induces single strand breaks may require only the final step, (c), of the UV-repair sequence. A uvr⁻ strain deficient in step (a) would still be mms⁺ since step (c) would be functional in such a strain. This scheme accounts for the UV sensitivity of the mms⁻ strain since any organism deficient in step (c) can not repair UV damage.

Howard-Flanders and Boyce (1964) suggested that some mutants unable to repair UV damage should be recombination deficient. Mutants unable to repair breaks should not participate in a process which requires breakage and rejoining. The mms⁻ strain was unable to transform and, in contrast to its parental strain, could not produce SP01 phage from infectious DNA. Both transformation and phage production from SP01 DNA require recombination(Okubo, Strauss and Stodolsky, 1964). However, we can not tell whether mms⁻ is unable to recombine or merely unable to take up DNA.

Transformability was not completely eliminated in mms⁻ and the mms⁻ strain had some residual ability to repair UV damage(Fig. 2, 4). This residual ability may be accounted for by supposing that step (c) is only partially blocked or it may be that the repair processes are more complex than now supposed.

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