NOTES

Repair of Alkylated Bacteriophage T4 Deoxyribonucleic Acid by a Mechanism Involving Polynucleotide Ligase

MARIAN W. BALDY,¹ BARBARA STROM, AND HARRIS BERNSTEIN

Molecular Biology Program, Microbiology Department, University of Arizona, College of Medicine, Tucson, Arizona 85721

Received for publication 31 December 1970

Methyl methanesulfate-induced lesions in bacteriophage T4 are repaired primarily by a mechanism involving polynucleotide ligase. Apparently, other recombinational and ultraviolet repair functions aren't involved.

It has been proposed that alkylation of deoxyribonucleic acid (DNA) occurs primarily at the 7' position of guanine and that this reaction leads to depurination followed by single-strand breakage (5). Brookes et al. (3) have reported that when phage T4 is treated with methyl methanesulfonate (MMS) about 600 alkylations per chromosome are required to give one phage lethal hit (37% survival). This suggests that after infection an active repair mechanism operates to remove most of the lesions.

In the current investigation, temperature-sensitive (*ts*) mutations in various early function genes were tested for sensitivity to MMS in order to identify which enzymes might be required for the repair of MMS lesions. Treatment with MMS was according to the following protocol. A 0.33-ml sample of 10 M MMS was added to 3.0 ml of phage at a titer of 5×10^7 phage/ml in phosphate-buffered saline, pH 7.2. At 3, 6, 9, and 12 min a 0.5-ml sample was transferred to 0.5 ml of 40% (w/v) sodium thiosulfate for 5 min to stop the reaction. A 0.5-ml sample of this mixture was then added to 0.5 ml of *Escherichia coli* B/5 in H-broth at a concentration of 10^8 to 10^9 bacteria/ml for 2 min. Inactivation, stopping the reaction, and adsorption were carried out at 37 C. After adsorption, 0.5 ml was diluted into 4.5 ml of chilled H-broth, and then an appropriate dilution was plated on *E. coli* S/6 at 35 C in one set of experiments and at 37 C in another. These temperatures are only partially permissive for most *ts* mutants. Repair of MMS lesions occurs in the initially infected bacteria on the plates.

Forty-four different strains were tested for MMS sensitivity by plating at 35 C. These included 40 *ts* mutants representing nine genes, the ultraviolet (UV) sensitive strains, T4 ν and T4 $\times$ (4), wild-type, and a revertant of *tsB20*. The inactivation curves for 38 of the *ts* mutations, representing eight genes (see legend to Fig. 1), wild-type, and T4 ν , fell entirely within the shaded area in Fig. 1.

As shown in Fig. 1, mutant *tsB20* which is defective in gene 30, the gene encoding polynucleotide ligase, was strikingly MMS sensitive. The dose required to give 37% survival was about a third of that required in the nonsensitive strains. The inactivation curve for a revertant of *tsB20* selected for ability to form plaque at 42 C is also shown. The revertant has an intermediate sensitivity, implying that it had not reverted to true wild-type. It was, nevertheless, substantially less sensitive than *tsB20* implying that the *ts* lesion itself, rather than an undefined background mutation in the *tsB20* strain, had caused the enhanced sensitivity. Another mutant defective in gene 30, *tsA80*, was also tested. The inactivation curve of this mutant is not shown in Fig. 1, but it fell along the lower edge and partly within the shaded area and thus indicated a marginal sensitivity. Finally, T4 $\times$ proved also to be strongly MMS sensitive (Fig. 1). All of the mutants which were tested by plating at 35 C were also tested at 37 C (except for T4 ν and T4 $\times$). Similar inactivation curves were obtained, and in no instance was a sharp increase in MMS sensitivity with the 2 C rise noted.

The enhanced sensitivity of *tsB20* implies

¹ Present address: 25 Oak Drive, Chico, Calif. 95926.

FIG. 1. Inactivation of phage T4 by MMS at 35°C. The inactivation curves of 38 *ts* mutants, wild-type, and T4v fell entirely within the shaded area. The 38 *ts* mutants were: gene 32 (G26, L67, L94, L170, P7, L171, P40); gene 39 (N41); gene 41 (A14); gene 42 (L40, L66, G13, G15, G23, G25B, A53, A64, A79, LB-1, LB-3); gene 43 (CB78, CB86, CB87, G37, G39, G40, L56, P25, CB123b, CB1245, CB87, CB124b, CB126); gene 44 (*ts*B110); gene 45 (*ts*L159); gene 46 (*ts*L109); gene 47 (A52, L86). The surviving fraction of all 38 *ts* mutants, T4v, and wild-type were averaged at each dose giving a composite inactivation curve (□). Other curves are for *ts*B20 (●), a revertant of *ts*B20 (○), and T4x (△).

that ligase action is a key step in the repair of MMS-induced lesions. It is not possible to interpret the MMS sensitivity of T4x in terms of a specific repair defect because the mutation (or mutations) to sensitivity in this strain have not been genetically or biochemically characterized.

The T4v mutant has been shown to lack an enzyme necessary for excision of thymine dimers

(6). Our results imply that this function is not essential for the repair of MMS lesions. The observation that none of the 13 *ts* mutations in the gene 43-encoded DNA polymerase showed significantly enhanced MMS sensitivity suggests that repair replication catalyzed by this enzyme is also not essential. Furthermore, it seems significant that two mutants tested here which are UV sensitive to an extent equal to or greater than *ts*B20 are not MMS sensitive. These are *ts*L67 and *ts*A52 (1), defective in genes 32 and 47, respectively. These genes have also been implicated in repair steps associated with recombination (2, 7).

We conclude from our results that methylation of DNA by MMS leads to single-strand nicks which are lethal unless repaired. The principal repair mechanism involves the gene 30 ligase and function(s) defective in T4x. Other gene functions implicated in recombination and repair of UV-induced lesions appear not to be part of this mechanism.

This work was supported by National Science Foundation grant GB-8760.

LITERATURE CITED

1. Baldy, M. W. 1970. The UV sensitivity of some early-function temperature-sensitive mutants of phage T4. *Virology* 40:272-287.
2. Bernstein, H. 1968. Repair and recombination in phage T4. I. Genes affecting recombination. *Cold Spring Harbor Symp. Quant. Biol.* 33:325-332.
3. Brookes, P., P. D. Lawley, and S. Venitt. 1969. Nature of alkylation lesions and their repair: significance for ideas on mutagenesis, p. 138-154. In G. E. W. Wolstenholme and M. O'Connor (ed.), *Mutations as cellular process*. J and A Churchill Ltd., London.
4. Harm, W. 1963. Mutants of phage T4 with increased sensitivity to ultraviolet. *Virology* 19:66-71.
5. Lawley, P. 1966. Effects of some chemical mutagens and carcinogens on nucleic acids. *Progr. Nucl. Acid Res. Mol. Biol.* 5:89-131.
6. Sekiguchi, M., S. Yasuda, S. Okubo, H. Nakayama, K. Suimada, and Y. Takagi. 1970. Mechanism of repair of DNA in bacteriophage. I. Excision of pyrimidine dimers from ultraviolet irradiated DNA by an extract of T4-infected cells. *J. Mol. Biol.* 47:231-242.
7. Tomizawa, J., N. Anraku, and Y. Iwama. 1966. Molecular mechanisms of genetic recombination in bacteriophage. VI. A mutant defective in the joining of DNA molecules. *J. Mol. Biol.* 21:247-253.