RECOMBINATION: DAMAGE AND REPAIR OF BACTERIOPHAGE GENOME[1]

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Brookes and Lawley (1961) demonstrated that bifunctional alkylating agents such as nitrogen and sulfur mustard form interstrand cross-links between complementary DNA strands in double-stranded DNA, thus inactivating double-stranded DNA-containing bacteriophage. This hypothesis is suggested by the fact that cross-linkage between two guanine residues can be detected by isolation of di-(guanin-7-yl) derivatives after treatment of DNA with the agent (Brookes and Lawley, 1961). As previously reported (Yamamoto and Naito, 1965; Yamamoto et al., 1966), bifunctional alkylating agents also inactivate bacteriophages containing single-stranded DNA and RNA, suggesting that inactivation may occur also by intrastrand cross-linkage in DNA or RNA.

When phage P22 was treated with nitrogen mustard (HN_2) and assayed on Salmonella typhimurium strain Q1 lysogenic for P22lb, a very high frequency of recombination between the damaged P22 and the prophage P22lb was found. These findings, which are described in this paper, strongly suggest that inactivation by intrastrand cross-linkage in DNA molecules initiates recombination. In a comparative study, a very similar enhancement of recombination by ultraviolet light(UV) is discussed.

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MATERIALS AND METHODS

Bacterial strains - Salmonella typhimurium, strain Q1 and its lysogenic strain Q1(P221b); S. typhimurium strain LT-2 (St) and its lysogenic strain St(P221b); and a P221b resistant mutant of Q1, Q1/221, were used in these studies.

Bacteriophages - The clear plaque-forming mutant (c_2) of Salmonella bacteriophage P22 was used for this study. P221bc+ was used for preparing the lysogenic strains (Yamamoto et al. 1966). Phage P22 has a short tail with hexagonal base plate and six spikes, whereas phage P221 has a long, flexible tail. Although they are serologically unrelated, they have a partial genetic homology (Yamamoto and Anderson, 1961; Yamamoto, 1964). In addition, Salmonella phages Fels 2, PIØ and Ph4 were also used for this study.

Chemicals - The following chemical agents were used in the present study: a monofunctional alkylating agent; ethylmethanesulfonate (EMS); two monofunctional mustard type alkylating agents, 2-chloroethylamine (NSC-10871) and 2-chloro-N, N-dimethylethylamine (NSC-1917); and one bifunctional nitrogen mustard, di-(2-chloroethyl)-methylamine, (HN_2) (NSC-762). Each alkylating agent was dissolved in M/15 phosphate buffered 0.1 M NaCl immediately prior to use.

Treatment of phage with nitrogen mustard - All manipulations were carried out at room temperature (25°C). One-tenth volume of alkylating agent solution in phosphate buffered saline (pH 7.0) was added to the phage suspension at a final concentration of 0.25 mg/ml and periodically, 0.1 ml samples were withdrawn by pipette and diluted 100-fold in the phosphate buffered saline containing 5% sodium thiosulfate to neutralize the further reaction of agent. After 30 minutes incubation at 25°C, the neutralized samples were diluted further, 0.25 ml aliquots of each diluted sample were added to 0.1 ml of indicator bacteria, 2 ml of soft agar (0.8% Difco Bacto agar in Difco nutrient broth) was added, and the mixture was poured over hard nutrient agar plates (1.5% agar). The number of plaques following overnight incubation at 37° served as the measure of

bacteriophage inactivation.

UV-irradiation - Gates Raymaster tubular 8-watt discharge bulbs, 12 inches long x 5/8-inch diameter, purchased from Arthur H. Thomas Co., were used. The lamp was fixed at a distance of 12 inches above the phage suspension to be irradiated. Irradiation was carried out with shaking, in a glass dish in which the liquid level did not exceed 1.0 mm.

RESULTS

When bacteriophage P22 was treated with nitrogen mustard, rapid inactivation of the phage was observed (Yamamoto and Naito, 1965; Yamamoto et al., 1966). If treated phage is assayed simultaneously on strains Q1 and Q1(P221b), survivors on the lysogenic strain Q1(P221b) are always higher than those on Q1 (Fig. 1). This reactivation may suggest that some defective P22 genome may grow in the presence of the prophage P221b as a helper virus. In order to test this possibility forty plaque clones were isolated from survivors on Q1(P221b) and assayed on Q1/221. All the clones can form plaques on Q1/221, thus ruling out the above possibility.

When clear plaque-forming mutants of P22 phage, P22c_2 were treated with nitrogen mustard and assayed on Q1(P221b), a very high frequency of turbid plaques (c+) was observed among survivors whereas no c+ plaque was found on Q1. Since it is well established that P22c_2 never reverts to the wild type P22c+ (Levine, 1957), the formation of c+ plaques on lysogenic strain must be a consequence of recombination between HN_2-damaged P22c_2 and the prophage P221b. Since P22 and P221b carry the genetic homology for the entire region containing c_1, c_2, c_3, g and h_{21} markers (Yamamoto, 1964), one possible explanation for the reactivation could be repair by recombination. A typical experiment on the kinetics of inactivation of phage P22c_2 and c+ formation among survivors is illustrated in Fig. 1. The c+ formation increases greatly as survivors decrease. At optimal conditions the c+ formation reached about 30% in comparison

with 0.2% in the untreated control.

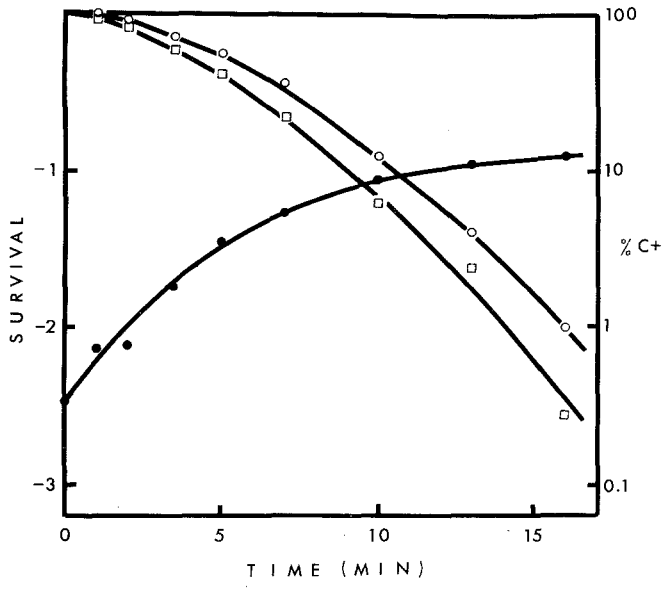

Fig. 1 Inactivation by nitrogen mustard (0.25 mg/ml) of Phage P22c_2 and c+ recombinant formation.
——O——O——: assayed on Q1(P221b).
——□——□——: assayed on Q1.
——●——●——: c+ recombinant on Q1(P221b).
Both ordinates are expressed on a log scale.

Using St and St(P221b) for assaying HN_2-damaged P22c_2 all the preceding observations were confirmed with almost identical results. However, if lysogenic strains for other Salmonella phages, Q1(Fels 1) and Q1(Fels 2), were used for assaying HN_2-damaged P22c_2 neither reactivation nor c+ formation was observed. Therefore, it is concluded that the reactivation and c+ formation from HN_2-damaged P22c_2 must occur in the lysogenic strain carrying the prophage, P221b, which is genetically related to P22. As we expected, when Q1(P221b) was used for assaying HN_2-damaged Salmonella phages (Fels 2, PIØ and Ph4) unrelated to P221b, neither reactivation nor c+ formation was observed.

As we reported previously (Yamamoto and Naito, 1965, Yamamoto et al., 1966), monofunctional mustards (2-chloroethylamine and 2-chloro-N, N-dimethylethylamine), in concentrations ranging from 1 to 10 mg/ml, did not inactivate phage P22 during 20 hrs exposure. These agents also had no effect on c+ formation. In addition, another monofunctional agent, EMS, at 0.5 M for 3 hr had no effect on phage P22. Thus, it appears likely that c+ formation is associated with inactivation resulting from cross-linkage on DNA molecules.

Table 1. Inactivation by nitrogen mustard (0.25 mg/ml) of phage P22c_2 and frequencies of recombinant types.

Survival	$\frac{\text{Recombinant}}{\text{survivors}}$ X 100 (%)		
(%)	Total c+	Heterozygote (c+/c_2)	complete c+
100	0.55	0.55	-
65.8	3.37	2.45	0.56
48.6	4.0	3.47	0.53
31.7	5.96	5.38	0.57
26.6	7.1	6.0	1.1
10.0	9.0	5.4	3.6
4.2	8.5	4.1	4.4
2.4	12.0	4.1	7.9
0.6	18.1	7.9	14.5

While studying c+ formation in P22 on Q1(P22lb), very high frequencies of heterozygote (c+/c_2) plaques were observed. As shown in Table 1, counts for c+/c_2 heterozygote plaques were very high at the beginning of inactivation whereas counts for complete c+ plaques remained very low. However, c+ formation overcomes heterozygote formation later in the inactivation kinetics. This

observation raises the possibility that single damage on one strand of a DNA molecule is responsible for the heterozygote formation but damage of both strands of DNA by multiple hits (two or more) is required for the formation of complete c+ plaques. Since monofunctional alkylating agents had no effect on this system, heterozygote formation can be explained by the assumption that damage and "recombination-repair" of the phage genome is due to intra-strand cross-linkage on one strand of a DNA molecule.

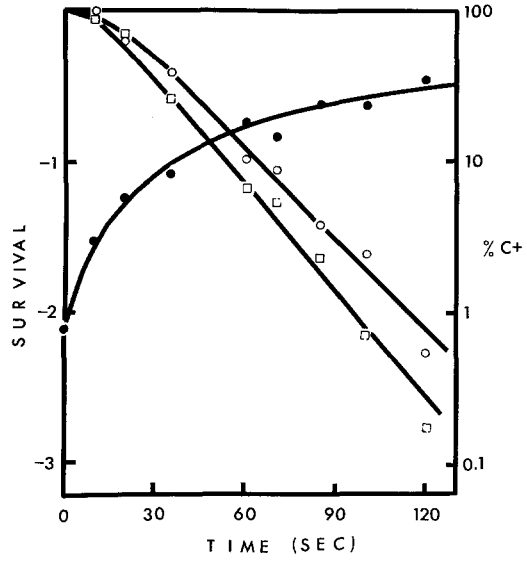

Fig. 2 Inactivation by UV of phage P22c_2 and c+ recombinant formation.
——○——○——: assayed on Q1(P221b).
——□——□——: assayed on Q1.
——●——●——: c+ recombinant on Q1(P221b).
Both ordinates are expressed on a log scale.

UV induces intrastrand thymine dimers in a DNA molecule (Beukers and Berends, 1960). This is analogous to intrastrand cross-linkage by HN_2. For this reason, the effect of UV was also tested on P22c_2 and compared with HN_2. As shown in Fig. 2, strikingly similar results for c+ formation and reactivation were obtained. Moreover, frequencies of the heterozygote formation induced by UV were very similar to those induced by HN_2. These findings sug-

gest that the mechanisms of damage and recombination-repair of the phage genome by HN_2 are similar to those by UV.

DISCUSSION

The wild type and UV-resistant mutant of E. coli strains are able to repair not only UV- but also HN_2-damaged DNA whereas UV sensitive mutants do not repair these damages (Howard-Flanders and Theriot, 1966; Kohn et al., 1965; Brookes, personal communication). This information also suggests the formation of intrastrand cross-linkage by HN_2 since both types of damage are similarly repaired.

As we reported previously, HN_2 inactivates single stranded bacteriophage at about the same rate as double stranded DNA phage, indicating considerable intrastrand cross-linkages (Yamamoto and Naito, 1965; Yamamoto et al., 1966). The frequency of heterozygote plaque formation by HN_2 treatment was very high (about 4-8%) at 50% inactivation even though only one marker, c_2 region, was studied. Moreover, the efficiency of heterozygote formation by HN_2 treatment is about the same as that by UV. Therefore, the formation of intrastrand cross-linkage by HN_2 may be of major significance for repair.

Although protein is reactive with HN_2, using indirect methods it has been suggested that damage by HN_2 of DNA is responsible for the inactivation of phage (Loveless, A. and Stock, 1959; Brookes and Lawley, 1961; Yamamoto and Naito, 1965; Yamamoto et al., 1966). The results in this paper present the most direct evidence that bifunctional alkylating agents act directly on DNA.

REFERENCES

Beukers, R. and Berends, W., Biochim. Biophys. Acta, 41, 550 (1960).
Brookes, P. "Chemotherapy of Cancer, Proc. Interntl. Sypo. at Luguano Ed. Plattner, P. A." pp. 32-43, Amsterdam: Elsevier Pub. Co. (1964).
Brookes, P. and Lawley, P. D., Biochem. J. 80, 496 (1961).
Brookes, P. and Lawley, P. D., Biochem. J. 80, 138 (1963).
Howard-Flanders, P. and Theriot, L. Genetics 53, 1137 (1966).
Kohn, K. W., Streigbigel, N. H., and Spears, C. L., P.N.A.S. 53, 1154 (1965).
Loveless, A. and Stock, J. C., Proc. Roy. Soc. London B. 150, 423 (1959).
Yamamoto, N., Science 143, 144 (1964).
Yamamoto, N. and Anderson, T. F., Virology 14, 430 (1961).
Yamamoto, N. and Naito, T., Science 150, 1603 (1965).
Yamamoto, N., Naito, T. and Shimkin, M. B., Cancer Res. 26, 2301 (1966).
Yamamoto, N. and Weir, M. L., Virology 28, 168 (1966).