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Synergistic Action of Bisulfite and Dimethylsulfoxide on Bacteriophage Lambda

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A synergistic action of sodium bisulfite and dimethylsulfoxide on bacteriophage lambda was found. A combination of 1 M sodium bisulfite, pH 5, and 3% dimethylsulfoxide showed a strong phage-inactivating effect, whereas 1 M sodium bisulfite alone or 3% dimethylsulfoxide alone was without effect. It was shown that both bisulfite and dimethylsulfoxide must be present simultaneously in the incubation mixture to achieve the inactivation. The phages that had been treated with a mixture of bisulfite and dimethylsulfoxide were shown to have lost their ability to transfer the viral deoxyribonucleic acid (DNA) to the host bacteria. It was also observed that a DNA sample isolated from the treated phages was fully active in transfection assay. From these results, it was concluded that the target of the reagents was the coat protein of the phage.

Keywords——bisulfite; dimethylsulfoxide; synergistic effect; bacteriophage lambda; inactivating effect

During our continuing studies on the mutagenic and inactivating actions of bisulfite on bacteriophage lambda,[1-4] we have found that dimethylsulfoxide (DMSO) has a synergistic effect on the phage-inactivating activity of bisulfite. Since bisulfite has become a standard reagent for inducing CG-to-TA transition mutations in microorganisms[5-7] and DMSO is a solvent in common use, we though it important to investigate this synergism in more detail. We describe here the results of this investigation.

Materials and Methods

The phage strains used were λpapa and λcI857Sam7. The indicator bacteria were *Escherichia coli* C600 for λpapa and Ymel for λcI857Sam7. *E. coli* C600(P1) was a strain possessing a restriction endonuclease for λcI857Sam7.

Preparation of both ^{32}P-labeled and unlabeled phages was done as described previously.[4] The activity of phage to transfer its DNA to the host bacterium was measured by the method of Scandella and Arber[8] as modified in our previous work.[4] Transfection activity of DNA was determined as described previously,[4] based on the method of Wackernagel.[9]

The reagents used were commercial reagent-grade chemicals.

Results and Discussion

When bacteriophage lambda was treated with 1 M sodium bisulfite containing 3% DMSO at pH 5 and 37°C, a rapid loss of its plaque-forming activity was observed (Table I). Treatment of the phage with bisulfite alone or with DMSO alone did not cause such a rapid inactivation. The rate of inactivation (logarithm of change in survival per unit time) was dependent on the concentrations of both bisulfite and DMSO; the rate increased with increase of the reagent concentrations (examined at 0.1—1 M sodium bisulfite and 1—5% DMSO). The optimum pH for the inactivating action of the reagents was 5 in the pH range of 4.2 to 7.3; at pH 7.3 the rate was approximately 1/5 of that at pH 5. At 23°C, the rate of inactivation by 1 M sodium bisulfite +3% DMSO at pH 5 was 1/3 of that at 37°C. Solvents other than DMSO were also examined for possible synergism with bisulfite; neither *N,N*-dimethylformamide nor hexamethylphosphoramide showed any effect on the phage at 3% concentration in combi-

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Table I. Inactivation of Bacteriophage Lambda by a Synergistic Action of Bisulfite and Dimethylsulfoxide[a)]

Reagent	Surviving fraction after treatment with the reagent(s)		
	Time of treatment (min)		
	0	10	20
$NaHSO_3$+DMSO in buffer	1	0.019	0.0003
$NaHSO_3$ in buffer	1	—	0.91
DMSO in buffer	1	—	0.99

a) A stock solution of λpapa (1×10^{10} PFU/ml), 0.1 ml, was mixed with 2 ml of 1 M sodium bisulfite ($NaHSO_3$)–1 M sodium citrate (pH 5.0) containing 3 %(v/v) dimethylsulfoxide (DMSO). A sample was taken up immediately from the mixture to determine the zero-time titer. The mixture was incubated at 37°C and the titer of phage at the desired period of incubation was determined as described previously.[4)] For control experiments, the incubations were done in 1 M $NaHSO_3$–1 M citrate ($NaHSO_3$ in buffer) and in 1 M NaCl–1 M citrate in the presence of 3% DMSO (DMSO in buffer).

nation with 1 M sodium bisulfite at pH 5 and 37°C. When the phage was treated first with bisulfite and then, after the removal of the bisulfite, with DMSO, no significant inactivation was observed. Similarly, treatment first with DMSO and then with bisulfite did not cause inactivation. Therefore, both bisulfite and DMSO must be present simultaneously in the incubation mixture to achieve this inactivation. Since bisulfite reacts with DMSO to give dimethylsulfide (identified by boiling point and nuclear magnetic resonance (NMR) measurements; similar reactions between thiols and DMSO have been reported[10)]), dimethylsulfide was examined for its effect on phage lambda. The treatment of phage with 1% dimethylsulfide (pH 5, 37°C) for 30 min, however, did not have any marked effect (survival, 0.76). We incubated a mixture of sodium bisulfite (1 M) and DMSO (5%) (pH 5, 37°C) for 15 h to complete the chemical reaction, then we added the phage to the mixture and incubated it for a further 20 min. No significant inactivation of the phage was observed (survival, 0.86). These results indicated that bisulfite and DMSO themselves were the reactive species towards the phage. The possibility of free radicals as the reacting species appeared unlikely because addition of hydroquinone (0.01 M and 0.1 M), a radical scavenger, to the bisulfite–DMSO mixture did not prevent the inactivation.

In order to determine the target of the inactivating action of the reagents, the ability of phage to transfer the phage DNA to the host bacteria, and the ability of phage DNA to transfect the spheroplast of *E. coli* were investigated. As Fig. 1 shows, the amount of DNA transferred to the bacteria decreased in parallel with the decrease of the plaque-forming activity. We have not attempted to determine whether this decrease is due to the loss of adsorption on the bacteria or to that of the DNA-injecting ability. The transfection activity of DNA, in contrast, was retained during the treatment of phage with bisulfite plus DMSO (Table II). These results clearly showed that the target for inactivation was the coat protein of the

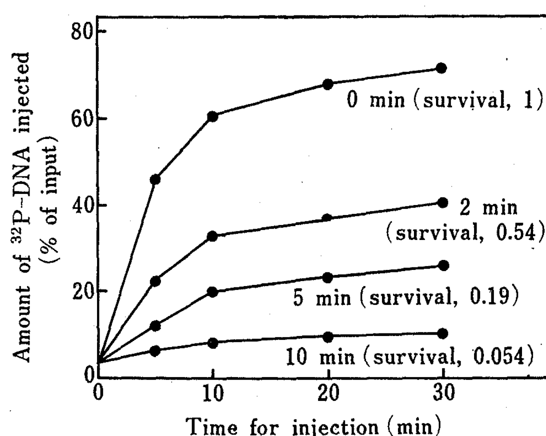

Fig. 1. Transfer of DNA from Bacteriophage Lambda to Host Bacteria

^{32}P-Labeled λcI857Sam7 was inactivated by treatment with 1 M sodium bisulfite plus 3% DMSO. The medium containing the inactivated phage was diluted to terminate the reaction and incubated with *E. coli* C600(P1) at an m.o.i. of 1 to achieve the adsorption and the injection. Aliquots were withdrawn at desired periods and the cold 2 % perchloric acid-soluble radioactivities were determined. The radioactivities represented the amounts of DNA injected into the host.[4)]

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Table II. Transfection Activity of DNA isolated from Phage Lambda treated with a Mixture of Bisulfite and Dimethylsulfoxide[a]

Incubation mixture	Time (min)	Surviving fraction	Transfection activity		
			Number of clear plaques formed (A)	Number of turbid plaques formed (B)	A/B
$NaHSO_3$+DMSO in buffer	0	1.0	65	63	1.03
			159	139	1.14
	10	0.005	83	79	1.05
			117	154	0.76
Control	0	1.0	147	127	1.15
			88	79	1.11
	10	0.79	138	132	1.05
			130	128	1.02

a) Transfection activity was measured as the activity of DNA from treated phage (λcI857Sam7, forming clear plaques) relative to that of an internal standard DNA (λpapa, forming turbid plaques). When there is a loss due to the treatment, the value A/B in this table should decrease.
Incubations were with 1 M $NaHSO_3$+3% DMSO in 0.5 M citrate, or with 1 M NaCl in 0.5 M citrate (Control), both at pH 5 and 37°C. The spheroplasts and the indicator bacteria used in this experiment were *E. coli* Ymel.

phage. In line with this conclusion, no increase was observed in the mutation frequency for clear plaque mutants during the treatment with bisulfite plus DMSO.

The chemistry of this synergism is not clear. A possible mechanism is the cleavage of disulfide linkages of proteins by attack of sulfite ion.[11] The role of DMSO would be to disrupt the protein conformation[12] to promote this sulfitolysis.

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