BISULFITE MUTAGENESIS IN BACTERIOPHAGE T4

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BISULFITE is employed as a preservative in foods and pharmaceuticals. Chemical studies of its interactions with nucleic acid components indicate that it reacts with uracil and cytosine in ways which might be mutagenic (SHAPIRO and WEISGRAS 1970; SHAPIRO, COHEN and SERVIS 1970; HAYATSU *et al.* 1970). Two preliminary assays, one in the bacterium *Escherichia coli* (MUKAI, HAWRYLUK and SHAPIRO 1970) and one in bacteriophage lambda (HAYATSU and MIURA 1970), revealed some bisulfite mutagenicity. In both instances, however, a maximum mutagenic response was observed within very short treatment intervals, no further mutagenicity being observed thereafter. The results with *E. coli* showed that only those mutants believed to revert at sites containing guanine:cytosine (G:C) base pairs responded to bisulfite; several mutants probably reverting at adenine:thymine (A:T) base pairs did not respond. Using the T4*rII* system, we have been able to demonstrate a very rigorous specificity of bisulfite mutagenicity for G:C base pairs. (Note, however, that T4 employs the cytosine analogue 5-hydroxymethylcytosine.) Furthermore, both inactivation and mutagenicity are strictly proportional to bisulfite concentration and to time of treatment.

We employed standard methods for the analysis of mutation in T4 (DRAKE 1970). Stocks of various well characterized T4*rII* mutants (DRAKE and MCGUIRE 1967; DRAKE 1963) were first diluted into 0.2% Bacto tryptone + 0.5% NaCl, and then 10-fold either into freshly prepared unbuffered $NaHSO_3$ solutions, or into 0.1 M sodium acetate buffer, at 37°C. The pH of both solutions was 5.0 both before and after incubation. Buffer alone was neither inactivating nor mutagenic, nor did it alter the effects of bisulfite treatment when included in the bisulfite solution. Total viable particles were assayed on BB cells, and r^+ revertants were assayed on KB cells.

Both inactivation and induced reversion of *rUV55* were studied as functions of bisulfite concentration (Figure 1) and time of treatment (Figure 2). Inactivation exhibits single-hit kinetics, with a rate constant of 0.85 M^{-1} hr^{-1} over 0 to 0.9 M and 0 to 4 hr. For this particular *rII* mutant, revertants were induced at the rate of 2.8×10^{-6} M^{-1} hr^{-1}. In stationary phase *E. coli*, on the other hand, using 1 M bisulfite at pH 5.2, a maximum induced mutant frequency was observed within the first treatment interval (0.5 hr), and no further increase occurred over the next 1.5 hr (MUKAI, HAWRYLUK and SHAPIRO 1970). No killing was observed even after 2 hr of treatment. In bacteriophage lambda, a maximum induced mutant frequency was observed by 1.5 hr, and no further increase occurred over the next 1.5 hr (HAYATSU and MIURA 1970). Inactivation was observed in the lambda

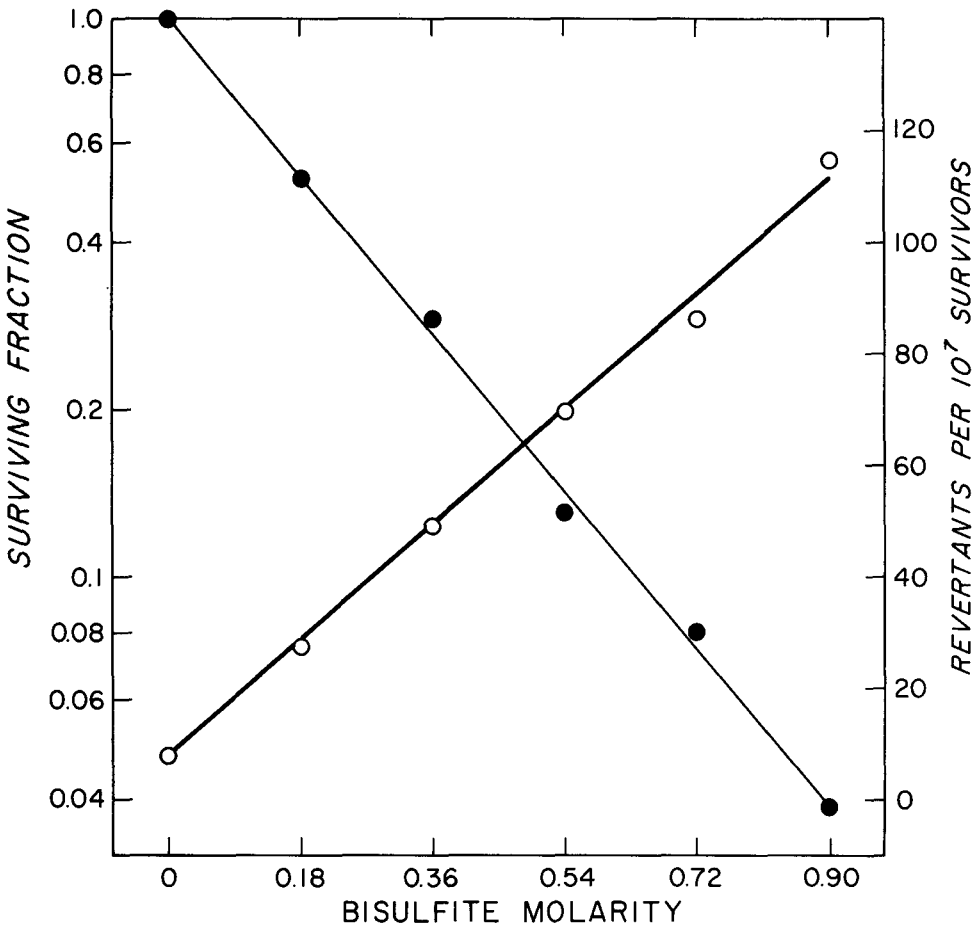

FIGURE 1.—Inactivation (-●-) and reversion (-○-) of T4*rUV55* as a function of $NaHSO_3$ concentration (treatment interval, 4 hr).

system, the single hit rate constant being 0.68 M^{-1} hr^{-1}, but inactivation in the buffer control (2.3 M NaCl + 1 M sodium phosphate, pH 5.6) occurred nearly (63%) as rapidly (based on a single treatment point), and the similarity of the T4 and lambda inactivation rates is therefore presumably coincidental.

Bisulfite mutagenesis exhibits a very marked allele specificity. When a variety of T4*rII* mutants were treated with 0.9 M bisulfite for four hours (Table 1), only those mutants were reverted by bisulfite which were also reverted by hydroxylamine. These mutants contain G:C base pairs at the reverting site, and can revert by G:C → A:T transitions. Mutants which can revert by A:T → G:C transitions (that is, are reverted by 2-aminopurine but not by hydroxylamine) did not respond significantly to bisulfite, in agreement with the *E. coli* results (MUKAI, HAWRYLUK and SHAPIRO 1970). Frameshift (fs) mutants (reverted by proflavin and not by base analogues) also failed to respond significantly. A number of mu-

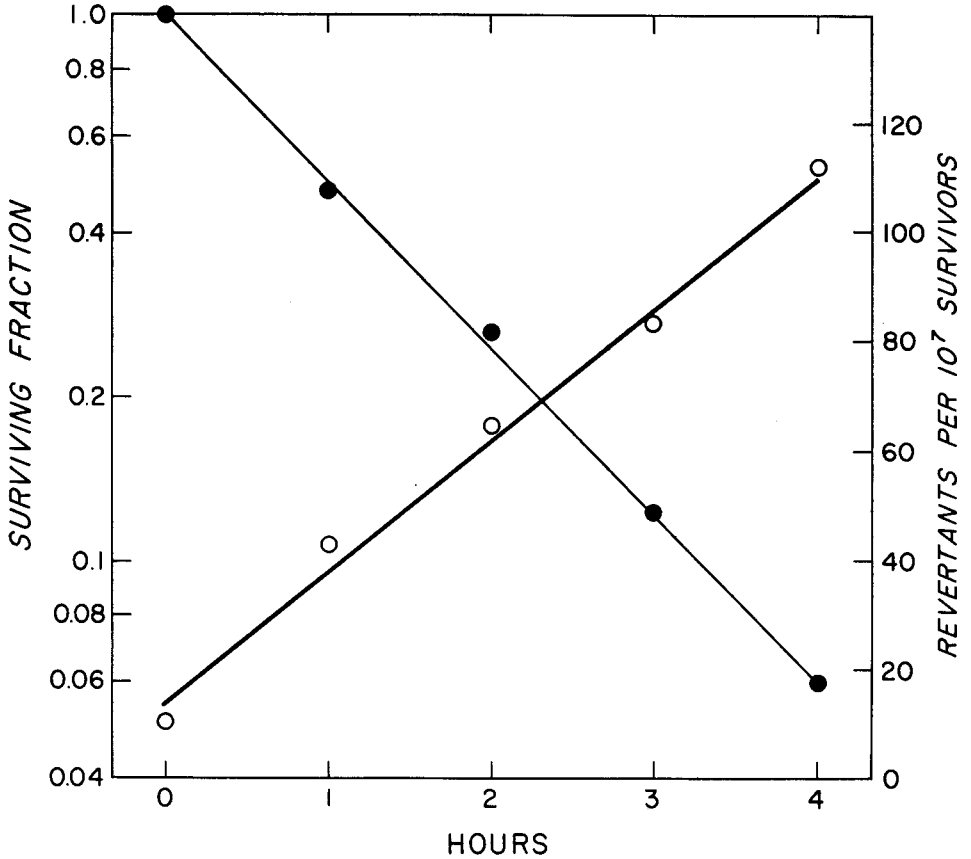

FIGURE 2.—Inactivation (-●-) and reversion (-○-) of T4*rUV55* as a function of time of treatment with 0.9 M $NaHSO_3$.

tants which revert spontaneously, but are not induced to revert by base analogues or by proflavin, also failed to respond to bisulfite. At least one of these mutants, *rSM94*, probably reverts from a G:C base pair by transversion (tv = purine: pyrimidine → pyrimidine:purine), since it is reverted by cytosine deprivation, which mutates G:C but not A:T base pairs (personal communication from R. BALTZ, E. GREENING and M. MOOGK). Bisulfite therefore not only mutates G:C sites very specifically, but also probably produces G:C → A:T transitions exclusively.

The contribution of lethal mutations to bisulfite inactivation can be estimated from the data of Table 1. Using the average G:C mutation rate, together with values of 35% for the G:C content and 180,000 for the number of base pairs in T4 DNA, the number of lethal hits per genome turns out to exceed the number of mutational hits by about 13-fold. Assuming that the T4*r* genes comprise 2% of the genome, we estimate that bisulfite would produce about 0.002 *r* mutations per lethal hit. By this criterion, bisulfite is a moderately strong mutagen; approximate corresponding values are 0.0002 for ultraviolet irradiation, 0.002 for

TABLE 1

*Bisulfite reversion of T4*rII *mutants*

Mutant	Base pair at mutant site	Revertants per 10^7 survivors Control	Treated
rUV7	G:C	1.1	10
rUV48	G:C	0.82	14
rUV55	G:C	8.7	110
rUV256	G:C	4.5	51
rUV363	G:C	2.5	21
rUV16	A:T	0.65	0.87
rUV30	A:T	0.091	0.13
rUV73	A:T	11	11
rUV304	A:T	2.7	4.9
rUV354	A:T	0.82	0.99
rUV6	(fs)	3.1	5.7
rUV28	(fs)	2.4	2.5
rUV58	(fs)	1.3	1.8
rUV113	(fs)	2.4	2.9
rUV353	(fs)	17	20
rSM18	?	2.6	3.2
rSM94	(tv;G:C)	1.1	2.4
rUV34	?	68	71
rUV61	?	1.5	1.8
rUV74	(tv;A:T)	3.4	1.7

nitrous acid, 0.01 for ethyl methanesulfonate, and 0.02 for hydroxylamine (DRAKE 1966). In terms of mutations per mole-hour, bisulfite is about as effective as nitrous acid applied at pH 5.3 (FREESE 1959), but is about 100 times weaker than nitrous acid applied at pH 3.7 (TESSMAN 1962).

Could bisulfite mutagenicity be detected in materials presented for human consumption? Using *rUV55* as a tester strain, a sodium bisulfite residue of 0.01 M (about 0.1%) would double the typical background revertant frequency in about 30 hr, with little inactivation. Furthermore, the strictly linear kinetics and concentration dependence of mutation and inactivation observed with *rUV55* make this system more suitable than others thus far developed.

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