The Mutagenic Specificity of Sodium Bisulfite

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Summary: Reversion studies with Escherichia coli mutants indicate that sodium bisulfite specifically induces mutations in only those mutants which are cytosine-guanine at the mutant site.

A base specific mutagen is of considerable value for the investigation of chemical mutagenesis. Chemical studies on the reaction of hydroxylamine with nucleic acid constituents (1), as well as biological studies with free single (2) and double (3) stranded bacteriophages have led to the inference that the major mutagenic effect of this mutagen is due to the alteration of cytosine (4, 5). While the mutational pattern for hydroxylamine has been in general agreement with the notion that cytosine-guanine to thymine-adenine base-pair transitions are chiefly induced by its reaction with cytosine, recent in vivo experiments with phage-infected host bacteria (6) and with bacterial mutants (7, 8) have indicated that hydroxylamine is also capable of producing thymine-adenine to cytosine-guanine transitions. Thus, the specificity of hydroxylamine for cytosine alone in mutagenicity studies remains to be established.

Recently, several authors (9-11) have reported on the chemical

reaction of sodium bisulfite with uracil and cytosine derivatives. They have shown that this chemical could deaminate cytosine to uracil without affecting adenine or guanine. One author (9) suggested that sodium bisulfite should be useful as a reference mutagen. The present report is concerned with mutational studies

Table 1 Characterization of E. coli mutants tested

Strain	Mutant Designation	Type	Assigned Change (wild type-mutant)
K12	A23 try$^-$	Transition	CG - TA
	A46 try$^-$	Transition	CG - TA
	A58 try$^-$	Transition	CG - TA
	A78 try$^-$	Transversion	GC - TA
15	A38 arg$^-$	Transition	TA - CG
	B23 cys$^-$	Transition	TA - CG
	H-2 ilva$^-$	Transition	TA - CG
	B14 his$^-$	Transition	TA - CG
	S-3 leu$^-$	Transition	TA - CG
	S-9 leu$^-$	Transition	TA - CG
	A11 leu$^-$	Transition	TA - CG
	S13 leu$^-$	Transition	TA - CG
	A23 leu$^-$	Transition	TA - CG
	A53 leu$^-$	Transition	TA - CG
	A17 ser$^-$	Transition	TA - CG
	S25 ser$^-$	Transition	TA - CG
	A12 try$^-$	Transition	TA - CG
	S-1 met$^-$	Transition	CG - TA
	S-8 pro$^-$	Transition	CG - TA
	S-8 ser$^-$	Transition	CG - TA
	S15 ser$^-$	Transition	CG - TA
	S19 ser$^-$	Transition	CG - TA
	S20 ser$^-$	Transition	CG - TA
	B38 his$^-$	Deletion	Deletion
	B-7 try$^-$	Deletion	Deletion
	B16 try$^-$	Deletion	Deletion

in E. coli which indicate that sodium bisulfite is a cytosine specific mutagen.

Materials and Methods: The strains of E. coli K12 and 15 used are listed in Table 1, together with relevant descriptive information. The type of genetic alteration assigned to the K12 mutants are from the amino acid replacement data of Yanofsky et al (12), while those assigned to the strain 15 mutants are deduced from the reversion experiments of Mukai and Troll (8).

Stationary growth phase cultures were used. Five ml of an overnight broth culture were centrifuged and washed once in saline. The washed pellet was resuspended in 5 ml of reaction mixture which was 1 M for sodium bisulfite in 0.2 M acetate buffer at pH 5.2. Control samples were resuspended in buffer alone at the same pH. At zero time and after 30 min incubation at 37°, sodium bisulfite and buffer were removed by centrifugation, and appropriate aliquots plated for titer and revertant colonies. The media and plating procedures have been described previously (8). Only large revertant colonies were counted after 36 hours incubation at 37° to exclude small suppressor type reversions.

Results and Discussion: Initial experiments to determine the optimal conditions for mutagenesis were performed with the CG mutant A38 arg^- and the results are shown in Table 2. There is no increase in the number of revertants after 30 min treatment at pH 5.2. Further, no killing of cells was observed under these conditions. Experiments using 1 M sodium bisulfite in 0.2 M phosphate buffer at pH 6, pH 7 and pH 8 showed that, only at pH 6 was there a significant increase in the number of revertants.

Table 2 Frequency of A38 arg$^-$ revertants induced by 1 M sodium bisulfite at pH 5.2

Agent	Frequency of revertants / 10^8 cells			
	0 min	30 min	60 min	120 min
Control buffer	22	18	23	22
Sodium bisulfite	20	125	94	100

However, at this pH, 20% of the cells were killed. Therefore, all subsequent work was done at pH 5.2 and 30 min exposure. These results are presented in Table 3.

Only those mutants identified as CG at the mutant site show an increase in reversion frequency. Since all of the TA and deletion mutants of strain 15 tested did not revert, the data for these mutants are omitted. In addition, reversion experiments with four UAG amber mutants of K12 were negative. We conclude from our observations that sodium bisulfite is specific for CG mutants alone.

While this manuscript was in preparation, Hayatsu and Miura (13) have reported that sodium bisulfite was mutagenic for the lambda phage and proposed that the mutagenicity may be due to CG to TA transitions. Studies on the chemical modification of phage DNA and yeast RNA have confirmed that bisulfite catalyzes the specific deamination of cytosine to uracil at the nucleic acid level (10, 13). The present data, in conjunction with the reported chemical data

Table 3 Frequency of reversions induced in E. coli mutants after 30 min exposure to 1 M sodium bisulfite at pH 5.2

Mutant	Assigned change at mutant site	Frequency of revertants / 10^8 cells	
		Control	Sodium bisulfite
A23 try$^-$	TA	1.2	2.8
A46 try$^-$	TA	5.2	4.2
A58 try$^-$	TA	0.5	1.1
A78 try$^-$	TA	11.6	11.6
A38 arg$^-$	CG	18.0	125.0
B23 cys$^-$	CG	2.6	80.1
H-2 ilva$^-$	CG	12.9	37.9
B14 his$^-$	CG	20.4	75.2
S-3 leu$^-$	CG	10.7	74.3
S-9 leu$^-$	CG	4.7	24.7
A11 leu$^-$	CG	38.3	163.9
S13 leu$^-$	CG	6.3	41.1
A23 leu$^-$	CG	6.6	20.5
A53 leu$^-$	CG	12.7	54.6
A17 ser$^-$	CG	20.1	45.1
S25 ser$^-$	CG	1.0	20.6
A12 try$^-$	CG	1.9	26.7

strongly indicate that sodium bisulfite causes mutations solely by its modification of cytosine, and that it should be useful as a cytosine specific reference mutagen.

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