THE MUTAGENIC ACTION OF SODIUM BISULFITE

Hikoya Hayatsu and Akiko Miura

Faculty of Pharmaceutical Sciencies,
University of Tokyo, Bunkyo-ku, Tokyo 113, Japan
and
Department of Chemistry, National Institute of
Health of Japan, Shinagawa-ku, Tokyo 141, Japan

Received February 26, 1970

Summary. This paper describes the mutagenic activity of sodium bisulfite toward phage λ. The frequency of mutation of the c gene of λ was increased about 10 times as much as that of spontaneous mutation by treatment of the phage with 3 M $NaHSO_3$ solution of pH 5.6 at 37° for 1.5 hrs. The mutagenesis could be related to the cytosine derivative specific reaction of sodium bisulfite.

In our current studies on the interactions of chemicals with nucleic acids and their components, we have discovered that sulfite ions react with a variety of pyrimidine bases[1-3]. Sodium bisulfite forms an addition compound with uracil, 5,6-dihydrouracil-6-sulfonate[2]. Cytosine and sodium bisulfite form an unstable adduct, dihydrocytosine-6-sulfonate, which is hydrolyzed to give 5,6-dihydrouracil-6-sulfonate. Uridine and cytidine have been found to undergo similar addition reactions with sodium bisulfite[3]. 5-Methylcytosine is converted into thymine by treatment with bisulfite[3]. These reactions occur at 37° and at pH around 6. The dihydrocytidine-6-sulfonate readily regenerates cytidine in its neutral solution. The 5,6-dihydrouridine-6-sulfonate is convertible into uridine on treatment with weak alkali. No reaction has so far been detected with other major nucleosides and nucleotides when they were treated with sodium bisulfite under similar conditions.

From these findings, it may be expected that cytosine (and 5-methylcytosine) residues in DNA will react with sodium bisulfite. Such reactions,

if they take place in vivo, may exhibit mutagenic or inactivating action on the organisms whose genetic informations are encoded in their DNA.

A mutant of phage λ which possesses a mutation at the c genes forms a "clear" plaque instead of a normal "turbid" one. By measuring the induction of clear mutations of phage λ mutagenic action of sodium bisulfite was found.

Materials and Methods

E. coli K12 used as the indicator for scoring c mutants of λh was N14-4[4] which is a derivative strain of W3623 try^- gal^- sm^r (Lederberg). General methods of handling of phage and media used were described in a previous paper[5]. 0.5 ml of solution of sodium bisulfite or control buffer and 0.01 ml of suspension of phage with the titer of 2.3×10^{10} were mixed and incubated at 37°. After various times, an aliquot was taken, diluted and plated to measure the mutation frequency. The sodium bisulfite solution was prepared by mixing 3 M $NaHSO_3$ and 3 M Na_2SO_3 solutions in 3:1 (v/v) ratio. The pH was 5.6. The composition of the control buffer is 2.3 M in NaCl and 1 M in sodium phosphate buffer, the final pH being 5.6. Mutagenesis by bisulfite was compared with that by hydroxylamine The c mutation was induced by treatment of λ with 1 M solution of hydroxylamine for 6 hrs at pH 7.5 and 37°. Treated λ phages were plated with indicator bacteria to give about 1,000 plaques in one plate. The term, frequency of mutation, is used to indicate the frequency of mutants among surviving phages.

Results and Discussion

The numbers of plaques screened and that of mutants found are shown in Table I, together with the frequency of mutation with 95 % probability level. The frequency of c mutation increased as a function of time of treatment with bisulfite, and the maximum value was obtained by the treatment for 90 min. Longer treatment did not seem to further increase the frequency. The absence of the increase of mutation frequency by the

Table I Frequency of c mutation induced by various agents.

Agent	Treated time min	Survival of λ	No. of plaques screened	No. of mutants found	Frequency observed units 10^{-5}	Frequency with 95 % probability units 10^{-5}
Sodium bisulfite	0	1	206,000	52	25	19- 34
	60	1.4×10^{-1}	42,600	69	160	130-200
	90	4.8×10^{-2}	40,800	107	260	220-320
	180	2.0×10^{-3}	36,100	101	280	230-340
Control	90	2.2×10^{-2}	83,700	27	32	22- 47
Hydroxylamine	360	1.1×10^{-1}	23,100	98	420	340-510

incubation in the control buffer eliminates the possibility that the mutation might have been caused by factors other than the bisulfite ions such as pH or high salt concentration. The potency of sodium bisulfite as a mutagen is comparable to that of hydroxylamine under the test conditions employed.

From these observations, we conclude that sodium bisulfite possesses a mutagenic activity.

Little can be stated at this stage of work about the mechanism of the mutagenesis caused by the bisulfite treatment. A transition mutation, C-G $\longrightarrow$ T-A, would be brought about by bisulfite-modification of cytosine (and/or 5-methylcytosine[6]). A preliminary experiment has shown that DNA, when treated, in vitro, with $H^{35}SO_3^-$, does incorporate the ^{35}S into its molecule, and, furthermore, this label can be completely removed by treatment with alkali (pH 12, 37°, 1 hr). This observation is just as expected from the experiments carried out with cytosine. In addition, when the DNA, which had been treated with bisulfite and then with alkali, was hydrolyzed with perchloric acid and the hydrolysate analysed by paper chromatography, a spot of uracil was detected. Uracil was not detectable in the hydrolysate of DNA that had not been treated with sulfite. These observations are in favor of the view that the mutation is caused by the modification of cytosine (and/or 5-methylcytosine) by bisulfite.

Chemical mutagens often exhibit cancer inducing activities. It would be interesting to examine if bisulfite ions could cause cancer in some organism. Such studies are of importance because of the air pollution by sulfur dioxide and of the sulfite salts being used as the food additives.

Acknowledgements

We thank Professor T. Ukita of the University of Tokyo and Dr. S. Nojima, Head, Department of Chemistry, National Institute of Health of Japan, for their giving us the opportunity of carrying out the present work and for their kind encouragement throughout it. Thanks are also

due to Professor J. Tomizawa of the University of Osaka for his critical reading of the manuscript.

References

1) Hayatsu, H., J. Am. Chem. Soc., 91, 5693 (1969).
2) Hayatsu, H., Wataya, Y., and Kai, K., J. Am. Chem. Soc., 92, 724 (1970).
3) Hayatsu, H., Wataya, Y., Kai, K., and Iida, S., to be published.
4) Ogawa, H., Shimada, K., and Tomizawa, J., Molec. Gen. Genetics, 101, 227 (1968).
5) Miura, A. and Tomizawa, J., Molec. Gen. Genetics, 103, 1 (1968).
6) Ledinko, N., J. Mol. Biol., 9, 834 (1964).