Bisulfite Modification of Nucleotides: Mechanistic Consideration leading to improved Protocols of DNA Methylation Analysis

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Treatment of DNA and RNA with bisulfite at pH 5-6 deaminates cytosine (C), selectively, to give uracil (U). This reaction was discovered in 1970 by us[1] and by Shapiro and coworkers[2]. We also found that 5-methylcytosine (C^m) reacts only slowly in this treatment[1]. The biological role of C^m in DNA is now a focus of attention, for the methylation is associated with gene function control, often accompanying physiological changes of organisms, *e.g.*, cancer. The bisulfite treatment is a principal method for analyzing the methylation of DNA, as it can discriminate C^m from C (Fig. 1). Several years ago, we attempted to improve this analytical procedure. The conventional method at that time included treatment of DNA with 3-5 M sodium bisulfite at 50-60°C for 16-20 hrs. We knew that the rate of C-deamination is highly dependent on the bisulfite concentration in the reaction mixture, although the mechanism of this dependence is unknown. We then succeeded to prepare a high concentration (10 M) bisulfite solution, and used it for the methylation analysis of genomic DNAs. A protocol, with which the deamination can be completed within 40 min at 70°C, was established[3]. This new protocol has been evaluated by workers of other laboratories and is now recommended as a one to be used to improve both the efficiency and the reliability of bisulfite treatments to collect data on DNA methylation states[4].

References

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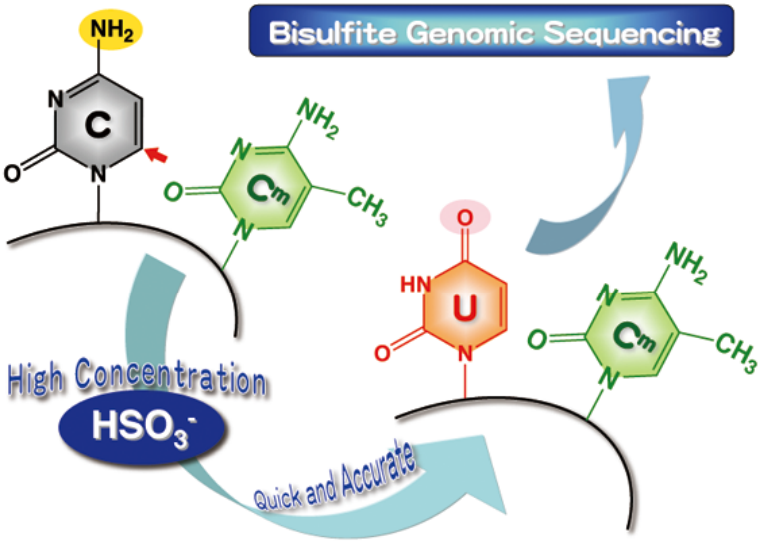

Fig. 1. Discrimination between cytosine and 5-methylcytosine by bisulfite modification.