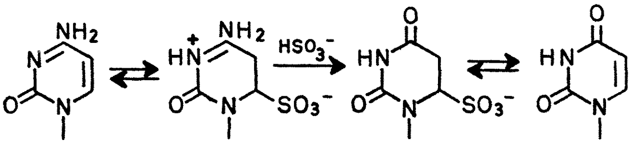
Dual Role of Bisulphite in the Deamination of Cytidine 5′-Phosphate

By HIKOYA HAYATSU* and MASANORI SONO

(*Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo, Japan*)

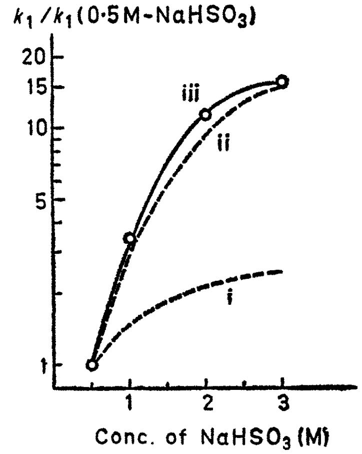
Summary Kinetic studies have shown that bisulphite (or sulphite) ion actively participates in the deamination of 5,6-dihydrocytosine-6-sulphonate nucleotide, that is, an unusual enhancement of the overall rate of cytosine-deamination with bisulphite can be obtained by increasing the concentration of bisulphite in the reaction mixture.

SODIUM BISULPHITE brings about the reaction,[1–3] cytosine nucleotide **(1)** $\rightleftharpoons$ **(2)** $\rightarrow$ **(3)** $\rightleftharpoons$ uracil nucleotide. Since this reaction occurs at 37° in nearly neutral solution, it constitutes an excellent method for deamination of cytosine residues in nucleic acids.[4,5] Mutagenesis with sodium bisulphite has also been found,[5,6] and proved to arise by base-pair substitution due to the modification of cytosine.[6] Furthermore, the bisulphite reaction has often been utilized in studies of the structure–function relationship of transfer RNA,[7,8] and also in tritium labelling of cytosine[9] and uracil[10] derivatives. During these investigations, we have become aware of the fact that, when the bisulphite concentration in the reaction mixture is increased, the rate of the deamination becomes unexpectedly high. Kinetic studies have now been carried out for the reaction, **(1)** $\rightleftharpoons$ **(2)** $\rightarrow$ **(3)**, and it has been found that bisulphite is actively participating not only in the first step, **(1)** $\rightleftharpoons$ **(2)**, but also in the hydrolysis, **(2)** $\rightarrow$ **(3)**.



(1) (2) (3)

The fractions of **(2)** in the equilibrium, **(1)** + $HSO^-_3 \rightleftharpoons$ **(2)**, at pH 5·8 and 37° were measured[3] for various combinations of the concentrations of **(1)** and sodium bisulphite. The value [**2**]/[**1**] $[HSO_3^-]$, [neglecting the dissociations of **(1)**, **(2)** and HSO_3^-] was found to be constant, 0·77 ± 0·03. The deamination was studied for solutions of pH 5·8 containing disodium cytidine 5′-phosphate (0·005 M), sodium bisulphite (0·5, 1, 2, and 3 M), and hydroquinone (0·0005 M), the hydroquinone having been added in order to avoid autoxidation of bisulphite. The amounts of **(3)** formed as a function of time at 37° were measured[3] and were found to follow pseudo-first-order kinetics. The bisulphite concentration did not appreciably decrease during the reaction, as determined by titration with iodine, and the pH value of the solution remained constant. The k_1 values (s^{-1}) found for the formation of **(3)** were as follows: 0·96, and 1·10 × 10^{-5} (0·5 M-$NaHSO_3$); 3·30, and 3·35 × 10^{-5} (1 M-$NaHSO_3$); 11·7, and 11·3 × 10^{-5} (2 M-$NaHSO_3$); 15·4 × 10^{-5} (3 M-$NaHSO_3$). Addition of 1 M-Na Cl in the 1M-$NaHSO_3$ reaction did not significantly affect the rate. The Figure shows relative k_1 values as a function of the concentration of bisulphite. In this Figure are also shown theoretical curves *i* and *ii* obtained by calculation on the basis of two possible reaction mechanisms: (**1**a) a mechanism in which bisulphite participates only in the equilibrium, (**1**) $\rightleftharpoons$ (**2**), and the second step is a simple hydrolysis, giving the expression, $k_1 = cK_E[HSO_3^-]/(K_E[HSO_3^-] + 1)$, where K_E is the equilibrium constant for (**1**) $\rightleftharpoons$ (**2**) and c is a constant (curve *i*); and (b) an alternative in which bisulphite actively participates in the step (**2**) $\rightarrow$ (**3**) as well, giving the expression, $k_1 = c'K_E[HSO_3^-]^2/(K_E[HSO_3^-] + 1)$, where c' is a constant (curve *ii*). The close fit of the observed curve (*iii*) to the curve (*ii*) strongly suggests that the mechanism (b) is valid.



FIGURE

Although the way in which bisulphite (or sulphite) ion participates in the hydrolysis of (**2**) remains to be elucidated, the present finding is important from the practical point of view. For example, for more rapid deamination of cytosine derivatives, it is greatly advantageous to maintain the concentration of bisulphite as high as possible. For such a purpose, use of ammonium bisulphite might be recommendable because not only is it more soluble than the sodium salt, but also ammonia accelerates the overall reaction, (**1**) $\rightarrow$ (**3**). Indeed, k_1 was found to be $38{\cdot}5 \times 10^{-5}$ (s^{-1}) for the reaction in 3·5 M-ammonium bisulphite.

We thank Professor T. Ukita of this Faculty for his encouragement, and Dr. Y. Kawazoe, of the National Cancer Center Research Institute, for valuable discussions.

(*Received, June 29th,* 1971; *Com.* 1094.)

[1] R. Shapiro, R. E. Servis, and M. Welcher, *J. Amer. Chem. Soc.*, 1970, **92**, 422.
[2] H. Hayatsu, Y. Wataya, and K. Kai, *J. Amer. Chem. Soc.*, 1970, **92**, 724.
[3] H. Hayatsu, Y. Wataya, K. Kai, and S. Iida, *Biochemistry*, 1970, **9**, 2858.
[4] R. Shapiro, B. I. Cohen, and R. E. Servis, *Nature*, 1970, **227**, 1048.
[5] H. Hayatsu and A. Miura, *Biochem. Biophys. Res. Comm.*, 1970, **39**, 156.
[6] F. Mukai, I. Hawryluk, and R. Shapiro, *Biochem. Biophys. Res. Comm.*, 1970, **39**, 983.
[7] Y. Furuichi, Y. Wataya, H. Hayatsu, and T. Ukita, *Biochem. Biophys. Res. Comm.*, 1970, **41**, 1185.
[8] Z. Kućan, K. A. Freude, I Kućan and R. W. Chambers, *Nature New Biology*, 1971, **232**, 177.
[9] K. Kai, Y. Wataya, and H. Hayatsu, *J. Amer. Chem. Soc.*, 1971, **93**, 2089.
[10] Y. Wataya, S. Iida, K. Kai, and H. Hayatsu, unpublished data.