Role of Bisulfite in the Deamination and the Hydrogen Isotope Exchange of Cytidylic Acid[1]

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Abstract: The mechanism of the bisulfite-catalyzed deamination and hydrogen isotope exchange of cytidine 5′-phosphate (CMP) was investigated by kinetic studies. The deamination which proceeds by the sequence, CMP + HSO_3^- ⇄ CMP-bisulfite adduct (CMP-SO_3^-) → UMP-bisulfite adduct (UMP-SO_3^-), showed pseudo-first-order kinetics in the presence of a large excess of bisulfite. Apparent rate constants for CMP → UMP-SO_3^- were determined, and the pH profile of the rate exhibited its maximum at pH 5. At pH 5 and 37° the rate constant, for example in 3 *M* sodium bisulfite solution, was 26.2×10^{-5} sec^{-1}. Examination of the relationship between the rate and bisulfite concentration has shown that the relative rate, k/k in 1 *M* $NaHSO_3$, is proportional to $K_E[NaHSO_3]^2/(1 + K_E[NaHSO_3])$, where K_E is the experimentally measurable equilibrium constant for CMP ⇄ CMP-SO_3^-. This relationship indicates that bisulfite ion participates in the hydrolysis of CMP-SO_3^- to UMP-SO_3^- in addition to the adduct-forming step CMP → CMP-SO_3^-. Deamination of cytidine by bisulfite action is similar to that of CMP with respect to pH profile and dependence on bisulfite concentration, although the reaction velocity is somewhat greater than that for CMP. Ammonium sulfite is a more preferable agent than sodium sulfite in order to obtain a faster reaction because the ammonium salt is more soluble than the sodium salt and ammonia exhibits an accelerating effect upon the deamination of CMP. Bisulfite-catalyzed hydrogen–deuterium exchange at the 5 position of CMP showed a pD maximum at 5.4 where the deamination concomitantly occurring was also maximal. Furthermore, dependence of the exchange rate on bisulfite concentration showed the same characteristic as that of the deamination. It was therefore concluded that two molecules of bisulfite were participating in both the deamination and the isotope exchange of a molecule of CMP.

Recently various aspects of bisulfite action on pyrimidine nucleosides have been revealed. Bisulfite ion adds across the 5,6 double bond of cytosine and uracil nucleosides, and the cytosine–bisulfite adduct is readily deaminated to give the uracil–bisulfite.[2-4] This reaction was considered to be responsible for the mutagenic activity of bisulfite,[5] and the bisulfite reaction has been utilized in chemical modification of tRNA.[6-9] Furthermore, in the bisulfite adduct of cytidine and uridine, the carbon–hydrogen bond at position 5 is labile so that the hydrogen can be easily replaced by deuterium or tritium.[10,11]

Current studies have disclosed another important aspect of bisulfite action on nucleotides, the free-radical reactions accompanying autoxidation. Thus, addition of sulfite radical occurs at the sulfur atom of 4-thiouridine[12] and 2-thiouracil[13] and at the olefinic double bond of isopentenyladenosine;[14] in dilute bisulfite solution (10^{-2}–10^{-3} *M*) under the catalysis of Mn^{2+} ion for the autoxidation, double-stranded DNA is rapidly altered in a manner that leads to chain cleavage in alkaline sucrose solution;[15] under similar conditions, transforming activity of *B. subtilis* DNA is lost with considerable velocity.[16]

These studies have at the same time provided implications for possible adverse effects of environmental sulfur dioxide. In particular, the mutagenic activity is of considerable concern from such a standpoint. There is evidence from genetic studies that the deamination of cytosine residues in DNA by bisulfite is indeed the cause of the mutagenesis.[17,18] In the bisulfite-catalyzed deamination of CMP[19] the first step (step 1), addition of bisulfite to CMP yielding CMP-SO_3^- (Chart I), is reversible and the equilibrium is reached very rapidly. Step 2, the deamination of CMP-SO_3^- to give the UMP-bisulfite adduct UMP-SO_3^-, is the rate-determining step. UMP-SO_3^- is stable at pH ≤ 7, although it liberates bisulfite on treatment with alkali giving UMP. The aim of the present research is to investigate the mechanism of the overall reaction CMP → UMP-SO_3^-. We carried out detailed kinetic studies on the bisulfite-catalyzed deamination of CMP. In addition, the bisulfite-catalyzed hydrogen isotope exchange at the 5 position of CMP was compared with the deamination with respect to dependence on pH and bisulfite concentration.

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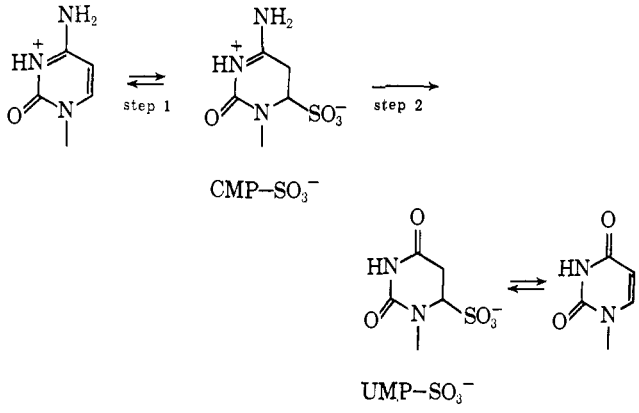
(2) R. Shapiro, R. E. Servis, and M. Welcher, *J. Amer. Chem. Soc.*, **92**, 422 (1970).
(3) H. Hayatsu, Y. Wataya, and K. Kai, *J. Amer. Chem. Soc.*, **92**, 724 (1970).
(4) H. Hayatsu, Y. Wataya, K. Kai, and S. Iida, *Biochemistry*, **9**, 2858 (1970).
(5) H. Hayatsu and A. Miura, *Biochem. Biophys. Res. Commun.*, **39**, 156 (1970).
(6) Y. Furuichi, Y. Wataya, H. Hayatsu, and T. Ukita, *Biochem. Biophys. Res. Commun.*, **41**, 1185 (1970).
(7) Z. Kućan, K. A. Freude, I. Kućan, and R. W. Chambers, *Nature (London), New Biol.*, **232**, 177 (1971).
(8) R. P. Singhal, *J. Biol. Chem.*, **246**, 5848 (1971).
(9) J. P. Goddard and L. H. Schulman, *J. Biol. Chem.*, **247**, 3864 (1972).
(10) K. Kai, Y. Wataya, and H. Hayatsu, *J. Amer. Chem. Soc.*, **93**, 2089 (1971).
(11) Y. Wataya and H. Hayatsu, *Biochemistry*, **11**, 3583 (1972).
(12) H. Hayatsu and M. Inoue, *J. Amer. Chem. Soc.*, **93**, 2301 (1971).
(13) M. Sono and H. Hayatsu, *Chem. Pharm. Bull.*, **21**, 995 (1973).
(14) H. Hayatsu, Y. Wataya, Y. Furuichi, and Y. Kawazoe, *Chemosphere*, **1**, 75 (1972).
(15) H. Hayatsu and R. C. Miller, Jr., *Biochem. Biophys. Res. Commun.*, **46**, 120 (1972).
(16) M. Inoue, H. Hayatsu, and H. Tanooka, *Chem.-Biol. Interactions*, **5**, 85 (1972).
(17) F. Mukai, I. Hawryluk, and R. Shapiro, *Biochem. Biophys. Res. Commun.*, **39**, 983 (1970).
(18) G. A. Summers and J. W. Drake, *Genetics*, **68**, 603 (1971).
(19) CMP represents cytidine 5′-phosphate; UMP, uridine 5′-phosphate; [s] represents concentration of total bisulfite buffer.

Chart I



Materials and Methods

CMP·Na_2 and cytidine were obtained from Takeda Chemical Industries and Kojin Co., respectively, the purity of each chemical being higher than 99% as checked by paper chromatography, paper electrophoresis, and ultraviolet absorption spectroscopy. All the other chemicals were of reagent grade available commercially.

Bisulfite solutions were always freshly prepared before use by dissolving in deionized water a mixture of sodium sulfite and sodium bisulfite in appropriate ratios to obtain desired pH values. When ammonium sulfite was employed as the sulfite source, the concentration of sulfite was determined by titration with iodine for the salt is highly hygroscopic. Ultraviolet absorption was measured by a Beckmann DU spectrophotometer, and nmr (100 MHz) by a Jeol-NM 4H-100 NMR spectrometer. The pD values reported in the present discussion were the readings on a pH meter calibrated with ordinary aqueous buffers.

Measurement of Rate of the Deamination. The reaction mixture contained 5×10^{-3} *M* CMP (or cytidine), 0.1–3 *M* bisulfite, and 5×10^{-4} *M* hydroquinone. The hydroquinone was added in order to minimize oxidative degradation of bisulfite. The mixture was placed in a tightly stoppered test tube and incubated in a water bath of an appropriate temperature. Aliquots were withdrawn at desired periods and diluted 50 times with 0.1 *M* potassium phosphate buffer (pH 7.0), and the solutions were allowed to stand at room temperature for 20 min in order to regenerate CMP from the unstable bisulfite adduct CMP–SO_3^-.[4] Absorbance at 271 nm was then determined which represented the amount of undeaminated CMP. A blank experiment in which CMP was omitted from the reaction mixture was carried out in each set of experiments. A pH check was routinely done before and after the incubation. Bisulfite solutions of pH ≥ 5 were generally stable and the pH change was less than 0.05 during the incubation. At pH <5 bisulfite solutions become less stable with increasing acidity, the pH change after 6 hr incubation at 37° being 0.2–0.5 toward smaller values. In typical runs bisulfite concentration was determined by titration with iodine and the decrease of the bisulfite due to oxidation was found generally not significant. Deamination accompanying the hydrogen–deuterium exchange at the 5 position of CMP was determined as described previously.[10]

Determination of the Equilibrium Constant for CMP + Bisulfite ⇄ CMP–SO_3^-. The equilibrium constant was defined as K_E = [CMP–SO_3^-]/[CMP][s], where the concentration of each component represents the sum of the concentrations for the dissociated and undissociated forms.[19] The K_E at 37° was estimated by using two independent methods.

(a) By Ultraviolet Absorption Spectroscopy. It is known that the equilibrium is attained very rapidly, within 1 min after mixing CMP with bisulfite at the pH region above 4.[2,4] To an aqueous bisulfite solution containing hydroquinone which has been maintained at 37° was added a solution of CMP, the final hydroquinone concentration being 5×10^{-4} *M*. The solution was mixed well and allowed to stand at 37° for 1 min. A portion of the solution was taken up and mixed with a large excess of 0.1 *N* HCl at room temperature. In 0.1 *N* HCl CMP–SO_3^- is stable and does not absorb uv light at greater than 260 nm.[4] Hence by measuring absorbance at 280 nm the CMP concentration can be determined.

A blank solution was prepared in each experiment subtracting CMP from the reaction mixture and worked up in the same manner as above. Since bisulfite absorbs uv light in acidic solution, the blank gave an $A_{280\ nm}$ value up to 30% of that of the test solution. The concentration of CMP–SO_3^- at the equilibrium was the difference between the CMP concentration determined as above and the initial CMP concentration.

(b) By Nmr Spectroscopy. A solution of CMP (0.8 *M*) and sodium bisulfite (0.8 *M*) in D_2O was subjected to nmr measurement at 37°. By comparing the signal strength of the 1′ proton of CMP (6.08 ppm, doublet, at pD 6.5) with that of the 5 proton of 5-monodeuterated CMP–SO_3^- (3.16 ppm, doublet, at pD 6.5), the molar ratio CMP/CMP–SO_3^- was determined. The recording was completed within 10 min after the solution was prepared.

Measurement of Hydrogen–Deuterium Exchange at the 5 Position of CMP. Nmr at 37° was recorded for the D_2O solution containing CMP and bisulfite. As the signals for proton 1′ (doublet) and proton 5 (doublet) are well resolved from each other at pD <7, the relative signal strengths can be determined with considerable accuracy using a method described elsewhere.[11] Thus, per cent exchange = 100 − [signal strength of proton 5/strength of proton 1′]100.

Results and Discussion

Deamination of CMP by Bisulfite. Dependence on pH and Bisulfite Concentration. Deamination of CMP with 2 *M* sodium bisulfite was performed at 37° and at various pH values. The decrease of the sum of CMP and CMP–SO_3^- was traced up to 90% decrease, the rate being analyzed by pseudo-first-order kinetics. The apparent rate constants found are plotted in Figure 1 as a function of pH. The bell-shaped profile shows the maximum at about pH 5. Rate constants of the deamination at pH 5.0 were determined at various bisulfite concentrations and the following results were obtained: concentration of sodium bisulfite, $k_{obsd} \times 10^5$ sec^{-1}; 0.1 *M*, <0.01; 0.5 *M*, 1.47; 1.0 *M*, 6.14; 2.0 *M*, 15.4; 3.0 *M*, 26.2. Salt effect appeared to be absent because the presence or absence of 1 *M* NaCl in 1 *M* $NaHSO_3$ at pH 5.8 did not affect the rate.

In order to evaluate these data it was necessary to determine the equilibrium constant for CMP + bisulfite ⇄ CMP–SO_3^-. Two spectroscopic methods were employed for this purpose. One method which utilizes uv is based on the fact that CMP–SO_3^- is stable in acid and does not absorb uv light at wavelengths where CMP does. The other method is to analyze nmr spectra taken directly on the reaction mixture and to obtain the molar ratio, CMP to CMP–SO_3^-. The values, K_E = [CMP–SO_3^-]/[CMP][s], thus obtained for various combinations of bisulfite and CMP concentrations are given in Table I. It can be seen that

Table I. Equilibrium Constants for the Adduct Formation between CMP and $NaHSO_3$ at 37°

Method	pH	K_E[a]	Initial concn of $NaHSO_3$	Initial concn of CMP
Uv	5.0	2.0	0.5	0.05
		2.3	0.5	0.1
		1.85	1.0	0.05
		1.87	2.0	0.1
Nmr	9.2[b]	0	0.8	0.8
	6.5[b]	0.5	0.8	0.8
	5.1[b]	1.8	0.8	0.8
	5.1[b,c]	2.1	0.8	0.8

[a] Defined as described in text. [b] pD. [c] Nmr was recorded 15 min, instead of 10 min in all other cases, after the start of the incubation.

the K_E values for a given pH are indeed approximately equal irrespective of varying concentrations of the re-

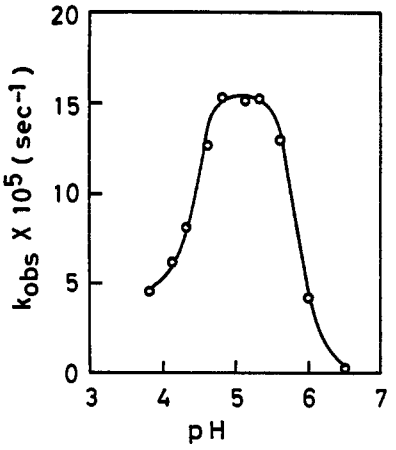


Figure 1. pH profile of bisulfite-catalyzed deamination of cytidine 5′-phosphate. Solutions containing 2 *M* Na_2SO_3-$NaHSO_3$ and 0.005 *M* CMP were incubated at 37°, and the rate constants were determined as described in the text.

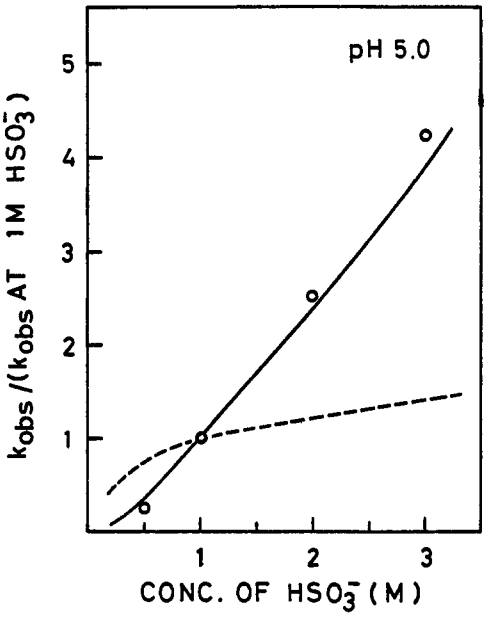


Figure 2. Relationship between bisulfite concentration and rate of deamination of CMP. Dashed line represents theoretical curve for mechanism 1 and solid line for mechanism 2. K_E value employed for the calculation was 2.0. See text for mechanisms 1 and 2.

acting components and of the method of measurement. Previously, Shapiro, *et al.*,[2] measured by nmr the per cent adduct for the equilibrium deoxycytidine + $NaHSO_3 \rightleftarrows$ dC-SO_3^-, and reported 100% for dC-SO_3^- at pD <6. The apparent difference between their results and ours could be due to the difference in the substrate.

An important consequence emerged by comparing relative rate constants at different bisulfite concentrations (Figure 2, circles) with those that can be predicted from theoretical considerations. Two mechanisms were considered. Mechanism 1 consists of a fast step of the equilibrium (step 1 in Chart I) and a slow step of simple hydrolysis of CMP-SO_3^- (step 2). This mechanism predicts $v = k_1$[CMP-SO_3^-] where v is the reaction velocity and k_1 a constant. Since K_E = [CMP-SO_3^-]/[CMP][s], it follows $v = \{k_1K_E[s]/1 + K_E[s]\}$([CMP] + [CMP-$SO_3^-$]) and therefore

$$k_{obsd} = k_1K_E[s]/(1 + K_E[s]) \quad (1)$$

Relative k_{obsd} values compared with k_{obsd} of 1 *M* bisulfite reaction were calculated using eq 1, and a theoretical curve thus obtained is shown in Figure 2 (dashed line).

In mechanism 2, the bisulfite molecule is participating in the slow step (step 2), *i.e.*, $v = k_2$[CMP-SO_3^-][s]. This mechanism predicts

$$k_{obsd} = k_2K_E[s]^2/(1 + K_E[s]) \quad (2)$$

The curve for relative k values calculated by eq 2 is also given in Figure 2 (solid line). It is obvious that mechanism 1 does not fit the experimental results but mechanism 2 does so satisfactorily. The same conclusion was reached when dependence of the rates on the bisulfite concentration was determined at pH 5.8 (see reference in footnote 1).

Effect of Temperature on the Reaction Rate. As the temperature was raised, the rate increased: k_{obsd} × 10^5 sec^{-1} (temperature/bisulfite concentration) at pH 5.8; 0.84 (0°/3 *M*); 10.4 (37°/2 *M*); 16.7 (37°/3 *M*); 38.5 (60°/2 *M*); 64.2 (60°/3 *M*).

Deamination of Cytidine. Cytidine was used as the substrate of the deamination in place of CMP. The optimum pH was found again at about 5, but the rate of the deamination was 30–50% larger than that of CMP at 37°. The results obtained are shown in Table II. In early studies we noted that the bisulfite-catalyzed deamination of cytidine proceeded to completion by a 21-hr reaction at pH 6, while the pH 5 reaction slowed down after several hours incubation, although the initial velocity was larger than that at pH 6.[4] This slow-down phenomenon is now attributable to the decrease in pH value due to extensive oxidation of bisulfite in the absence of antioxidant.

Table II. Rate Constants of Bisulfite-Catalyzed Deamination of Cytidine at 37°

Concn of $NaHSO_3$ (*M*)	k_{obsd} × 10^5, sec^{-1}		
	pH 4.5	pH 5.0	pH 5.8
1.0	6.7	10.2	7.2
2.0	17.8	22.7	16.7

Effect of Ammonia and Other Nucleophiles on the Deamination. As described above, increase of bisulfite concentration in the reaction mixture greatly accelerates the deamination. Since ammonium sulfite is more soluble in water than the sodium salt, the deamination of CMP was carried out at a high concentration (3.5 *M*) of ammonium sulfite. An unexpectedly high reaction velocity was observed and the rates including those for lower salt concentrations were found to be as follows: concentration of $(NH_4)_2SO_3/k_{obsd}$ × 10^5 sec^{-1}, at pH 5.8 and 37°, 3.5 *M*/38.5; 3.0 *M*/26.3; 2.0 *M*/14.8. When one compares the rate constants with those obtained by sodium bisulfite, one finds that the ammonium salt is significantly more effective than the sodium salt at the same bisulfite concentration. The effect of other amines was also examined by using sodium bisulfite solution supplemented with chloride of the amines. However, trimethylamine, guanidine, imidazole, or quinuclidine were found not to accelerate the deamination to a significant extent as ammonia does.

As an agent structurally related to bisulfite, methane-

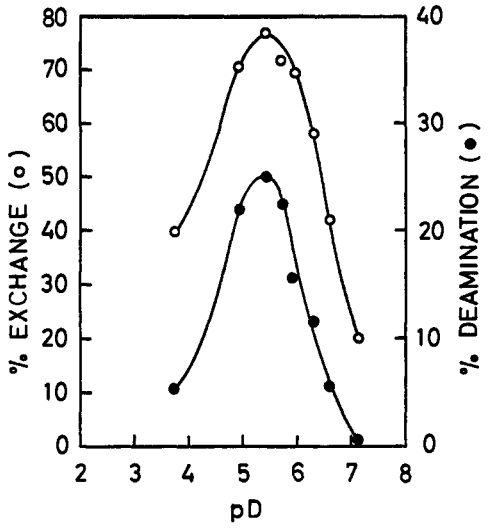


Figure 3. Similarity in pD profile between the deamination and the hydrogen–deuterium exchange. A D_2O solution containing 0.8 *M* CMP and 0.8 *M* $NaDSO_3$, of which pD had been adjusted by addition of DCl, was incubated at 37° for 3 hr. Nmr was recorded to determine the per cent exchange at the 5 position of CMP. A portion of the sample which had been taken up before the nmr measurement was used for determination of per cent deamination. See text for methods.

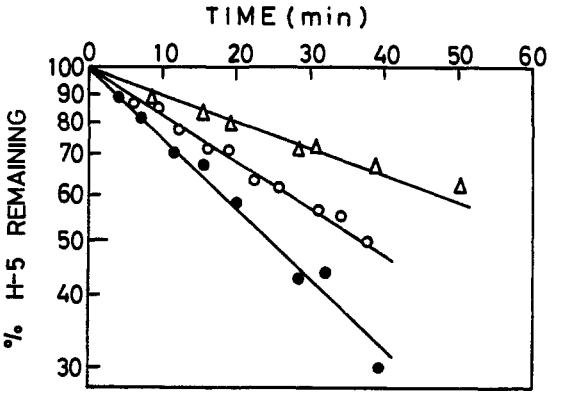


Figure 4. Time course of hydrogen–deuterium exchange at the 5 position of CMP as a function of bisulfite concentration. Condition A (△), 0.80 *M* CMP, 0.80 *M* $NaDSO_3$ at pD 5.39; condition B (○), 0.78 *M* CMP, 1.55 *M* $NaDSO_3$ at pD 5.49; condition C (●), 0.76 *M* CMP, 2.27 *M* $NaDSO_3$ at pD 5.56. Reaction temperature was 37°.

sulfonic acid was examined as to its ability to catalyze or accelerate the deamination of CMP. No deamination, however, was detected in 1 *M* CH_3SO_3H solution containing 5 m*M* CMP (pH 5, 37°), nor was any accelerating effect of supplementing 1 *M* CH_3SO_3H to 1 *M* $NaHSO_3$ upon the deamination.

Bisulfite-Catalyzed Hydrogen–Deuterium Exchange at the 5 Position of CMP. Dependence on pD and Bisulfite Concentration. Previously we have reported that sodium bisulfite catalyzes the hydrogen isotope exchange of CMP, and have investigated the exchange rate at the pH range of 7–9 where the deamination does not appreciably take place.[10,11] It was interesting to find when pD dependence of the exchange reaction was determined in a more acidic region that the optimum pD was 5.4 (Figure 3), because the deamination of the same reaction mixtures was optimal also at pD 5.4. Figure 3 shows the close similarity in the pD profile of the two different reactions. This finding has suggested that the two reactions may operate by a common mechanism.

Effect of bisulfite concentration on the hydrogen isotope exchange was therefore studied. This study was somewhat complicated by the fact that the concentration of CMP in the reaction mixture must be high enough to permit accurate nmr measurement, and that the formation of the adduct CMP–SO_3^- as well as the deamination concomitantly taking place consumes bisulfite thereby reducing its concentration. However, a semilog plot of per cent exchange showed a linear relationship against time of treatment up to about 40% reaction (Figure 4). In addition, the velocity of the exchange was dependent on bisulfite concentration. This can be interpreted as follows. The hydrogen isotope exchange may occur through the adduct CMP–SO_3^- under the action of bisulfite. Once the equilibrium between CMP and CMP–SO_3^- is reached the bisulfite concentration should remain approximately constant in spite of the fact that deamination of CMP–SO_3^- proceeds gradually. For example, simple calculation for the reaction mixture A, B, and C of Figure 4 shows that the bisulfite concentration at 20% deamination is still 95–98% of the zero-time concentration. A small decrease in the concentration of CMP–SO_3^- due to the deamination should not affect the reaction velocity since what we are measuring is the per cent exchange in the surviving fraction of CMP. Hence it is not surprising to have obtained pseudo-first-order kinetics. As Table III shows,

Table III. Effect of Bisulfite Concentration on Hydrogen–Deuterium Exchange at the 5 Position of CMP

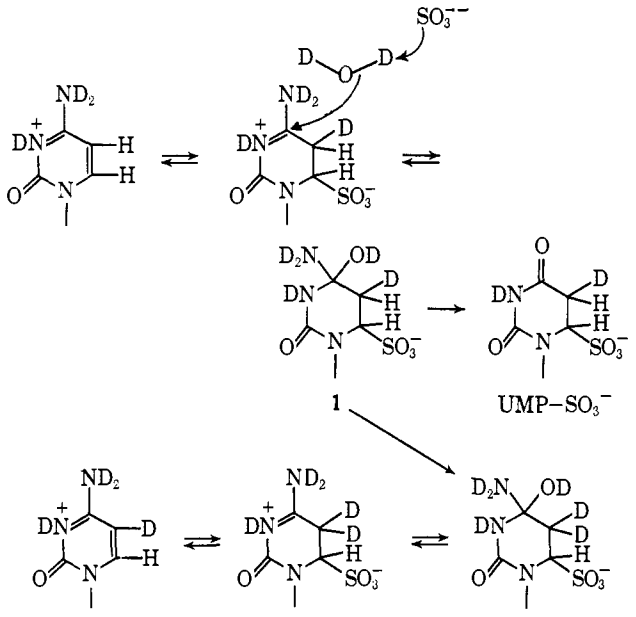
Condition[a]	Calculated concn at equilibrium (*M*)[b] D-s	CMP	CMP–SO_3^-	k_{obsd} found $\times 10^4$, sec^{-1}	Relative rate for H–D exchange of CMP–SO_3^-[c]
A	0.43	0.43	0.37	1.80	1.0
B	1.02	0.25	0.53	3.13	1.22
C	1.69	0.18	0.58	4.56	1.61

[a] The conditions A, B, and C correspond to those in Figure 4. [b] These are the zero-time values calculated on the assumption that the equilibrium is reached instantaneously. $K_E = 2$ was employed for the calculation. D-s represents concentration of total D-bisulfite buffer. [c] $(k_X/k_A)/([CMP\text{–}SO_3^-]_X/[CMP\text{–}SO_3^-]_A)$.

the relative rates for hydrogen–deuterium exchange of CMP–SO_3^- were dependent on the bisulfite concentration, in consistence with the mechanism in which bisulfite ion participates in the exchange of CMP–SO_3^-. This finding again supports the view that the deamination and the hydrogen isotope exchange proceed *via* a common intermediate.

Mechanism of the Reaction. Chart II represents one possible mechanism. The mechanism involves a common intermediate, **1**, for the two reactions formed by addition of hydroxide to the 4 position of deuterated CMP–SO_3^-. The role of a second bisulfite molecule in these reactions may be catalysis of the step by proton transfer. Ammonia may also function at this step in a similar manner. By introducing another electron-withdrawing group to carbon 4, the proton at the 5 position may become easily dissociable. Intermediate formation of 5,6-dihydrouridine-4,6-disulfonate, which may result by direct substitution of the amino group of

Chart II



$CMP-SO_3^-$ with sulfite, is unlikely since the disulfonate is known to be stable under similar conditions[12] and such a compound was not detected by paper chromatography and paper electrophoresis of the reaction mixture. Although it is not known whether the rate-determining step is the formation of **1** or the subsequent hydrolysis (or hydrogen exchange), the fact that the reaction is optimal at pH ~5 suggests that the protonated species of $CMP-SO_3^-$ and sulfite anion may be involved.

Previously an analogy was pointed out[4] between the bisulfite-catalyzed deamination of cytidine and the Bucherer reaction. In the Bucherer-type deamination of naphthylamine, however, the rate-determining step is the addition of bisulfite to the starting material.[20] Hydrolysis of the amino group of the resulting adduct corresponding to $CMP-SO_3^-$ is a fast step in contrast to the case of cytidine. Indeed, kinetic studies using 1-aminonaphthalene-4-sulfonic acid as the substrate have shown that the deamination rate is linear to the first order of bisulfite concentration.[21]

Comments on Mutagenesis by Bisulfite. Recently Summers and Drake[18] using bacteriophage T4 showed that the bisulfite-induced inactivation and reversion of G:C to A:T base pair are proportional to bisulfite concentration in the range of 0.18–0.9 *M*. On the basis of the results shown in Figure 2, in which the rate of deamination of CMP is approximately proportional to bisulfite concentration in the range of 0.2–3 *M*, their data appear to be consistent with the assumption that the observed rate of inactivation depends upon the rate of the bisulfite-catalyzed deamination of the cytosine residue (in T4 DNA 5-hydroxymethylcytosine is the constituent in place of cytosine).

Since $k_{obsd} = k_2K_E[s]^2/(1 + K_E[s])$, when the [s] is low enough so that $K_E[s] \ll 1$, the following correlation is obtained

$$k_{obsd} = k_2K_E[s]^2$$

In such a situation, lowering of bisulfite concentration, for instance, to 0.1 will result in a decrease of the rate of deamination of cytosine by a factor of 0.01. Hence at lower concentrations of bisulfite a proportional correlation will not be observed between the bisulfite dose and the rate of inactivation as well as that of mutation.

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(20) H. Seeboth, *Angew. Chem., Int. Ed. Engl.*, 6, 307 (1967).

(21) W. A. Cowdrey and C. N. Hinshelwood, *J. Chem. Soc.*, 1036 (1946).

Communications to the Editor

Stereochemical Nonrigidity in Seven-Coordinate Trihydridorhenium Complexes

Sir:

Although magnetic equivalence of ligand nuclei in seven-, eight-, and nine-coordinate complexes has often been attributed to fluxional behavior,[1-7] only for the eight-coordinate complexes $MH_4[P(C_6H_5)_2CH_3]_4$ and $MH_4[P(C_6H_5)_2C_2H_5]_4$ (M = Mo, W)[8a] and the seven-coordinate complex $Mo(NO)(S_2CNMe_2)_3$[8b] have both limiting fast- and slow-exchange nmr spectra been detected. We now report the first observation of this for seven-coordinate hydride complexes.[9] The complexes are $ReH_3(dppe)_2$[11] (dppe = $(C_6H_5)_2PCH_2CH_2P(C_6H_5)_2$) and $ReH_3(dpae)_2$[12a] (dpae = $(C_6$-

(1) S. C. Abrahams, A. P. Ginsberg, and K. Knox, *Inorg. Chem.*, 3, 558 (1964).
(2) E. L. Muetterties, *Inorg. Chem.*, 4, 769 (1965).
(3) J. Chatt and R. S. Coffey, *Chem. Commun.*, 545 (1966).
(4) E. L. Muetterties and C. M. Wright, *Quart. Rev., Chem. Soc.*, 21, 109 (1967).
(5) A. P. Ginsberg, *Chem. Commun.*, 857 (1968).
(6) J. Chatt and R. S. Coffey, *J. Chem. Soc. A*, 1963 (1969).
(7) E. L. Muetterties, *Accounts Chem. Res.*, 3, 266 (1970).
(8) (a) J. P. Jesson, E. L. Muetterties, and P. Meakin, *J. Amer. Chem. Soc.*, 93, 5261 (1971); P. Meakin, L. J. Guggenberger, W. G. Peet, E. L. Muetterties, and J. P. Jesson, *ibid.*, 95, 1467 (1973); (b) R. Davis, M. N. S. Hill, C. E. Holloway, B. F. G. Johnson, and K. H. Al-Obaidi, *J. Chem. Soc. A*, 994 (1971).
(9) The complexes π-$C_5H_5MoH(CO)_2L$ are formally seven-coordinate and stereochemically nonrigid. However, their permutational behavior is like that of a five-coordinate complex since in this respect the π-cyclopentadienyl group acts as if it takes up only one coordination position.[10]
(10) J. W. Faller and A. S. Anderson, *J. Amer. Chem. Soc.*, 92, 5852 (1970).
(11) M. Freni, R. Demichelis, and D. Giusto, *J. Inorg. Nucl. Chem.*, 29, 1433 (1967).
(12) (a) A. P. Ginsberg and M. E. Tully, to be submitted for publication. (b) All ^1H nmr spectra were run in a ~10% CS_2 solution with TMS as an internal standard.