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Modification of Adenine and Cytosine Derivatives with Bromoacetaldehyde

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Bromoacetaldehyde reacted with adenosine 5′-phosphate and cytidine more rapidly than chloroacetaldehyde to give 1,N^6-ethenoadenosine 5′-phosphate and 3,N^4-ethenocytidine, respectively. The pH-dependence of the rates of these reactions was similar to that of the chloroacetaldehyde modifications reported in the literature; *i.e.* the reactions proceed smoothly in the pH range of 5 to 7. When applied to polynucleotides, bromoacetaldehyde reacted with adenine and cytosine residues located in single-stranded regions but not with those in double-stranded regions. Guanosine 5′-phosphate reacted with bromoacetaldehyde at pH 7 but only very slowly at pH 5. Bromoacetaldehyde may be useful as a more reactive subtitute for chloroacetaldehyde.

Bromoacetaldehyde showed little mutagenic activity when assayed on *Salmonella typhymurium* TA 100.

Keywords——adenosine 5′-phosphate; cytidine; guanosine 5′-phosphate; bromoacetaldehyde; chemical modification; mutagenicity; polynucleotides; 1,N^6-ethenoadenosine 5′-phosphate; 3,N^4-ethenocytidine; 3,N^4-ethenocytosine

Since the discovery of the reactivity of chloroacetaldehyde with adenine and cytosine,[2] these reactions have found wide application in nucleic acid studies.[3] An advantage of this reaction is that the products, 1,N^6-ethenoadenine and 3,N^4-ethenocytosine, possess characteristic fluorescence properties which permit easy detection of these derivatives. Another feature of the reaction is its single-strand specificity for nucleic acid,[4] and this property was utilized in recent studies on the conformations of transfer ribonucleic acid (RNA)[5] and replicating deoxyribonucleic acid (DNA).[6]

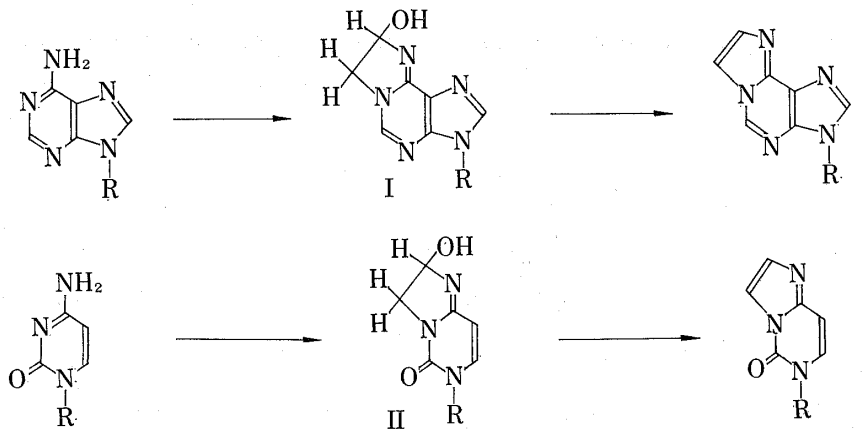

Chart 1. Bromoacetaldehyde Modification of Adenine and Cytosine Derivatives

1) Location: *Tsushima, Okayama 700, Japan.*
2) N.K. Kochetkov, V.N. Shibaev, and A.A. Kost, *Tetrahedron Lett.*, **1971**, 1993.
3) R.W. Thomas and N.J. Leonard, *Heterocycles*, **5**, 839 (1976).
4) K. Kimura, M. Nakanishi, T. Yamamoto, and M. Tsuboi, *J. Biochem.*, **81**, 1699 (1977).
5) L.H. Schulman and H. Pelka, *Biochemistry*, **15**, 5769 (1976).
6) T. Kohwi-Shigematsu, T. Enomoto, M. Yamada, M. Nakanishi, and M. Tsuboi, *Proc. Natl. Acad. Sci. USA*, **75**, 4689 (1978).

The reaction may be improved by using bromoacetaldehyde in place of chloroacetaldehyde, because the bromo compound is expected to be more reactive than the chloro compound. This paper deals with the reactions of bromoacetaldehyde with adenine and cytosine derivatives. A preliminary investigation on the reaction with guanosine 5′-phosphate is also described.

Experimental Procedures

Reagents——Chloroacetaldehyde was obtained from Tokyo Kasei Chemicals as a 50% aqueous solution. 1,N^6-Ethenoadenosine, 3,N^4-ethenocytidine, and poly(I)–poly(C) were products of PL Biochemicals. Poly(A), poly(C) and calf thymus DNA were purchased from Sigma. Other reagents were reagent grade commercial products.

Preparation of Bromoacetaldehyde——Method 1: This method is essentially the same as that previously described by Schukovskaya *et al.*[7] In our case, bromine (40.4 g) was cooled in an ice-salt bath, and vinyl acetate (21.7 g) was added dropwise to the stirred bromine with exclusion of moisture. The addition took 2.5 hr, and the reaction was allowed to proceed for a further period of 0.5 hr. Fractional distillation of the mixture gave (1,2-dibromoethyl)acetate: bp 70—74°/10 mmHg (lit.[7] 89°/16 mmHg); 38.2 g; 62%. The (1,2-dibromoethyl)acetate (10 g) was mixed with 10% HCl (10 ml) and the mixture was heated at 60° for 2.5 hr with stirring. The cooled mixture was neutralized by the addition of calcium carbonate and the product was separated from the salt by exhaustive extraction with ether. Bromoacetaldehyde hydrate was obtained from the ether solution by distillation: bp 90—98° (lit.[7] 90.5—92.5°); 4.7 g; 25%. The material solidified upon cooling and melted at room temperature. This material was used in the present study without further purification. When stored at −75°, it was stable for several months.

Method 2: Bromoacetaldehyde diethylacetal (8 ml) was mixed with oxalic acid (6 g) and the mixture was distilled. The fraction boiling at 100—112° was collected; this contained bromoacetaldehyde and some water.

Determination of the Ethenylating Activity of Halogenoacetaldehyde——A sample of the bromoacetaldehyde (0.20 g) was dissolved in water (3.80 ml). One volume of the solution was mixed with nineteen volumes of 0.5 M adenosine 5′-phosphate (an aqueous solution previously adjusted to pH 7), and the mixture was allowed to stand at room temperature for five days. A portion of the mixture was then dilute 10^4-fold with water and the fluorescence emission at 407 nm (excitation at 310 nm) was measured. Comparison of the fluorescence intensity with those of solutions containing known amounts of 1,N^6-ethenoadenosine gave the concentration of 1,N^6-ethenoadenosine 5′-phosphate formed in the test solution. (In a separate experiment, 1,N^6-ethenoadenosine 5′-phosphate was found to possess the same fluorescence intensity as 1,N^6-ethenoadenosine: thus, there was no change in the intensity when 1,N^6-ethenoadenosine 5′-phosphate was dephosphorylated by treatment with alkaline phosphatase.) Assuming that all the bromoacetaldehyde originally contained in the reaction mixture had been utilized in the ethenylation, the bromoacetaldehyde content of the sample was calculated. The ethenylating activity of chloroacetaldehyde was determined by the same procedure. The molar concentrations of the halogenoacetaldehydes reported in this paper are corrected for this ethenylating activity.

Time Course of the Reaction between Bromoacetaldehyde and Adenosine 5′-Phosphate——The reaction mixture contained 0.01 M adenosine 5′-phosphate, 0.5 M buffer, and bromoacetaldehyde. Incubation was at 37°. Aliquots (20 μl each) were withdrawn at desired times and applied to strips of Toyo filter paper No. 53. Immediately after drying the sample by blowing cool air, the strip was subjected to ascending chromatography using isobutyric acid–NH_3–H_2O (75: 1: 24) as the developing solvent. From the chromatogram thus obtained, the ultraviolet-absorbing spots corresponding to 1,N^6-ethenoadenosine 5′-phosphate (*Rf* 0.34) and to adenosine 5′-phosphate (*Rf* 0.41) were excised and eluted with water. The amounts of these nucleotides were determined by measuring the ultraviolet absorbances of the eluate. Molar extinction coefficients employed in the determination were 6.2×10^3 at 275 nm for 1,N^6-ethenoadenosine 5′-phosphate, and 15.4×10^3 at 260 nm for adenosine 5′-phosphate. Buffers used for studying the pH-rate profile were sodium acetate (pH 3—6), sodium phosphate (pH 6—7) and triethylammonium bicarbonate (pH above 7). The pH values of the reaction mixtures were measured both at the start and the end of the incubation.

The time course of the reaction of chloroacetaldehyde with adenosine 5′-phosphate was examined by the same method.

Modification of Poly(A) with Bromoacetaldehyde——A solution (1.2 ml) containing poly(A) (100 $A_{260\,nm}$/ml), 0.5 M sodium phosphate (pH 6.5) and bromoacetaldehyde (0.3 M) was incubated at 37° for 15 min. The modified poly(A) that precipitated was separated from the reagents by removing the supernatant solution, washed with ethanol and ether, and dried. For determination of the extent of modification, this material

7) L.L. Schukovskaya, S.N. Ushakov, and N.K. Galanina, *Izu. Akad. Nauk SSSR, Otd. Khim. Nauk*, **1962**, 1692; *CA*, **58**, 8891*g* (1963).

(6 A_{260} units) was digested with nuclease P_1 (0.1 g) by incubation at 50° and at pH 5 for 1.5 hr, and the nucleoside 5′-phosphates thus formed were fractionated by paper chromatography (solvent as described above) and quantitated spectrophotometrically.

Bromoacetaldehyde Modification of the Adenine Residue in DNA for Estimation of the Single-strand Specificity——Calf thymus DNA was denatured by heating it in aqueous solution at 100° for 10 min followed by rapid cooling with ice-water. The denatured DNA or the native DNA (2 mg/ml) was mixed with an equal volume of 1 M sodium phosphate, pH 7, which contained 0.12 M bromoacetaldehyde, and the mixture was incubated at 37°. Aliquots (0.2 ml) were removed from time to time, mixed with 0.05 M sodium phosphate (pH 7; 4 ml) and the fluorescence intensities at 407 nm (excitation at 310 nm) were recorded.

Preparation of 3,N⁴-Ethenocytosine——Cytosine (222 mg) was dissolved in hot water (6 ml). The solution was maintained at 37° and bromoacetaldehyde (0.5 g) was added to it. The pH was adjusted to 6.5 by addition of a few drops of 10 N NaOH, and the solution was allowed to stand at 37°. The progress of the reaction was monitored by thin-layer chromatography on cellulose (solvent; isopropanol–NH_3–H_2O, 7: 1: 2), and the pH was maintained at 6 to 6.5 by occasional addition of NaOH. After incubation for 42 hrs, more bromoacetaldehyde (0.25 g) was added and the mixture was incubated for a further 30 hrs. The mixture, in which the product had precipitated, was kept in a refrigerator overnight. The needle crystals in the mixture were collected by filtration, and washed with water, ethanol and ether: yield, 196 mg (73%). This material was subjected to elemental analysis without further recrystallization. *Anal.* Calcd for $C_6H_5N_3O$: C, 53.33; H, 3.73; N, 31.10. Found: C, 53.33; H, 3.65; N, 31.05. UV in H_2O: λ_{max} 258, λ_{min} 255; in 0.1 N HCl; λ_{max} 282, shoulder 245—257, λ_{min} 221 nm. MS (70 eV): *m/e* 135 (M^+), 107 (M^+-CO), 80 ($M^+-CO-HCN$), 53 ($M^+-CO-H_2C_2N_2$).

Bromoacetaldehyde Modification of Cytidine——The reaction mixture consisted of 0.01 M cytidine, 0.5 M buffer, and 0.12 M bromoacetaldehyde (or 0.15 M chloroacetaldehyde). During incubation at 37°, aliquots (20 μl each) were withdrawn and chromatographed on paper as described for the reaction of bromoacetaldehyde with adenosine 5′-phosphate. The chromatographic solvent was isopropanol–NH_3–H_2O (7: 1: 2). Two products, *i.e.* 3,N⁴-ethenocytidine (*Rf* 0.79) and the intermediate (presumably represented by structure II; *Rf* 0.62), were observable on the chromatogram in addition to the starting material (*Rf* 0.53). For estimation of the extent of reaction, the spot corresponding to cytidine was excised, eluted with 0.1 N HCl, and the amount of cytidine was determined from the absorbance at 280 nm (ε, 13.4×10^3). The decrease in the amount of cytidine as a function of time of treatment was used as a measured of the extent of reaction. Buffers employed for reaction at various pH values were the same as those for the reaction with adenosine 5′-phosphate.

Conversion of Intermediate II into 3,N⁴-Ethenocytidine——An aqueous solution of the intermediate, free of bromoacetaldehyde, was prepared by preparative paper chromatography (solvent as above), eluting the compound from the chromatogram with water, then evaporating the aqueous eluate to dryness, and redissolving the residue in water. The resulting solution was mixed with an appropriate buffer (sodium acetate, pH 5, or sodium phosphate, pH 7; the final concentrations were 0.2 M) and the mixture was incubated at a desired temperature. Aliquots were removed and subjected to paper chromatography to determine the extent of the conversion of II into 3,N⁴-ethenocytidine.

Single-strand Specificity of the Bromoacetaldehyde Modification of Cytosine——Poly(C) [or poly(I)–poly(C)], 1 mg/ml, was incubated with 0.12 M bromoacetaldehyde at 37° and pH 7 in 0.5 M sodium phosphate buffer. Aliquots (0.1 ml) were taken, diluted with 0.05 M sodium phosphate buffer (pH 7; 2 ml), and the absorbance at 300 nm was recorded. From the rise in $A_{300\ nm}$, the extent of the poly(C) modification was determined. For this purpose, the rise in $A_{300\ nm}$ in the cytidine-bromoacetaldehyde reaction was correlated with the extent of reaction determined as described under "Bromoacetaldehyde modification of cytidine."

Mutagenesis Assay of the Halogenoacetaldehyde——The assay was carried out by the method of Ames *et al.*[8] as modified by Yahagi *et al.*,[9] using *Salmonella typhimurim* TA 100 and measuring the reversion from His^- to His^+. In this process, the mixture containing bacteria and the halogenoacetaldehyde was incubated at 37° for a desired period before adding soft agar to plate the whole mixture on agar. "S-9"[8] was not used in this assay.

Results

Among several known procedures for preparing bromoacetaldehyde,[7,10,11] two were found to be satisfactory from a practical point of view. The first consisted of bromination of vinyl-

8) B.N. Ames, J. MacCann, and E. Yamasaki, *Mutation Res.*, 31, 347 (1975).
9) T. Yahagi, M. Nagao, Y. Seino, T. Matsushima, T. Sugimura, and M. Okada, *Mutation Res.*, **48**, 121 (1977).
10) E. Fischer and K. Landsteiner, *Ber.*, **25**, 2549 (1892).
11) H. Hibbert and H.S. Hill, *J. Am. Chem. Soc.*, **45**, 734 (1923).

Table I. Purity of Bromoacetaldehyde Samples as determined in Terms of the Ethenylating Activity

Method of preparation[a]	Bromoacetaldehyde content in the sample, calculated from the activity[b] (%)
1	78.6[c]; 78.4; 73.6
1	64.0[c]; 62.8; 62.4
1	97.6
2	30.0

a) Methods 1 and 2 are those described in "Experimental Procedures."
b) See "Experimental Procedures."
c) A 0.2 ml sample solution in water was mixed with 1.8 ml of the adenosine 5′-phosphate solution, whereas in other cases a 0.1 ml sample solution was mixed with 1.9 ml of the adenosine 5′-phosphate solution.

acetate followed by acid hydrolysis,[7] and the second utilized degradative distillation of bromoacetaldehyde diethylacetal by addition of oxalic acid.[10] The bromoacetaldehyde samples were checked for their ethenylating activity by incubation with a large excess of adenosine 5′-phosphate. The "purities" of four bromoacetaldehyde samples determined in this way are listed in Table I. This method for determining the ethenylating activity of the bromoacetaldehyde proved to be reproducible and dose-dependent. Although the second method of preparing bromoacetaldehyde was simple, the product was found to be relatively impure, and therefore we decided to use bromoacetaldehyde prepared by the first method in this work.

Reaction of Adenine Derivatives with Bromoacetaldehyde

The reaction of adenosine 5′-phosphate with bromoacetaldehyde was monitored by paper chromatography with an acidic solvent. In this procedure, the intermediate I present in the reaction mixture was transformed in 1,N^6-ethenoadenosine 5′-phosphate during chromatographic development. The amount of 1,N^6-ethenoadenosine 5′-phosphate plus adenosine 5′-phosphate recovered from the chromatogram was constant throughout the time course studied, indicating that the reaction proceeded stoichiometrically.

Wiewiorówski and his collaborators[12,13] have shown that an appreciable amount of intermediate I accumulates during the chloroacetaldehyde modification of adenosine. Because of the presence of the intermediate, monitoring of the present reaction by ultraviolet absorbance or by fluorescence emission proved to be difficult. When the extent of reaction was estimated from the rise in $A_{295\,nm}$ after dilution with 0.05 M sodium phosphate buffer (pH 7; ε value 3000 for 1,N^6-ethenoadenosine), the kinetics did follow a pseudo-first-order rate law. However, the half-life of the starting material measured in this way was about three times longer than that measured by the paper chromatographic method. This result may be explained by assuming that intermediate I does not significantly absorb light at 295 nm.

When the formation of 1,N^6-ethenoadenosine 5′-phosphate in the reaction was measured in terms of the flourescence emission of the etheno compound at 407 nm (excitation at 310 nm), the time required for 50% formation of the product was again about three times longer than that for a 50% decrease in the starting material as measured by the paper chromatographic method.

Figure 1 shows time courses of the disappearance of adenosine 5′-phosphate at various pH values. It can be seen that the rate of the reaction does not change greatly in the pH range of 4.6 to 6.6, but decreases outside this range. Lowering of the pH of the reaction mixtures during

12) J. Biernat, J. Ciesiolka, P. Górnicki, R.W. Adamiak, W.J. Krzyzosiak, and M. Wiewiórowski, *Nucleic Acids Res.*, **5**, 789 (1978).
13) W.J. Krzyzosiak, J. Biernat, J. Ciesiolka, P. Górnicki, and M. Wiewiórowski, *Polish J. Chem.*, **53**, 243 (1979).

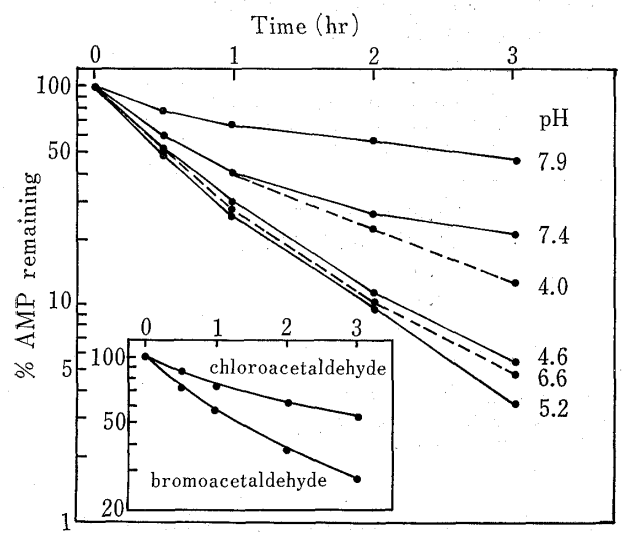

Fig. 1. Time Course of the Reaction between Bromoacetaldehyde and Adenosine 5′-Phosphate at Various pH Values

Reaction mixtures consisted of 0.01 M adenosine 5′-phosphate, 0.13 M bromoacetaldehyde and 0.5 M buffer. The incubation was at 37°. The inset compares the reactivities of bromo- and chloroacetaldehyde: 0.01 M adenosine 5′-phosphate, 0.07 M bromoacetaldehyde or 0.08 M chloroacetaldehyde, and 0.5 M sodium phosphate; at pH 7.0 and 37°.

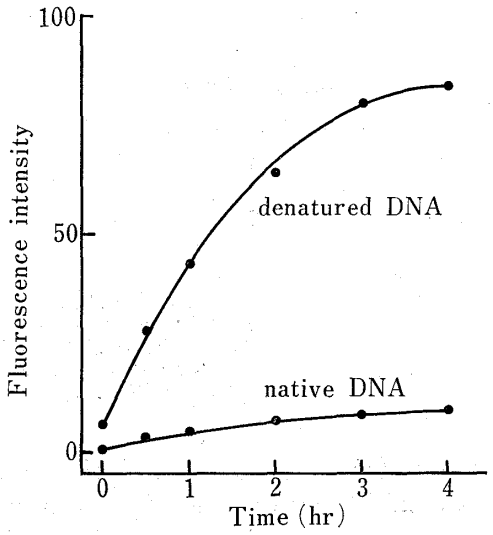

Fig. 2. Reaction of Bromoacetaldehyde with Adenine Residues in DNA

The reaction mixture, pH 7, contained DNA (1 mg/ml), bromoacetaldehyde (0.06 M) and sodium phosphate (0.5 M). Incubation was at 37°.

the incubation was less than 0.2, except at pH 7.9, when the pH fell to 7.3 after 3 hr. The inset of Fig. 1 compares the reactivities of bromoacetaldehyde and chloroacetaldehyde with adenosine 5′-phosphate: bromoacetaldehyde reacted about 2.5 times faster than chloroacetaldehyde.

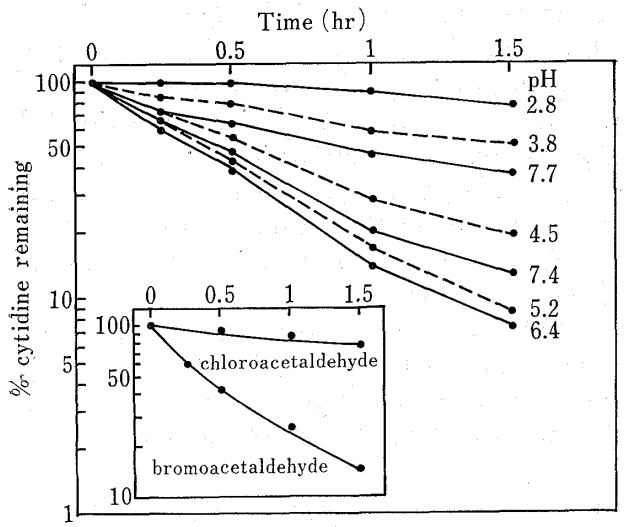

Fig. 3. Time Course of the Reaction between Bromoacetaldehyde and Cytidine at Various pH Values

Reaction mixture contained 0.01 M cytidine, 0.12 M bromoacetaldehyde and 0.5 M buffer. The incubation was at 37°. The reactions shown in the inset were carried out at pH 7.0 and 37° with 0.12 M bromoacetaldehyde or 0.15 M chloroacetaldehyde.

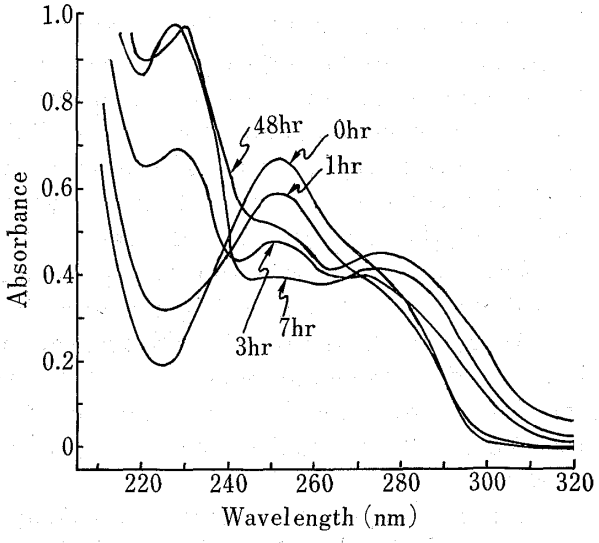

Fig. 4. Spectral Change during the Incubation of Guanosine 5′-Phosphate with Bromoacetaldehyde

The reaction mixture, pH 6.9, consisted of 0.01 M guanosine 5′-phosphate, 0.12 M bromoacetaldehyde and 0.45 M sodium phosphate. Incubation was at 37°. Aliquots were withdrawn at the indicated times, diluted 200-fold with 0.05 M sodium phosphate, pH 7, and the spectra were recorded.

Using bromoacetaldehyde, poly(A) can be modified quite rapidly. On treatment of poly(A) with 0.3 M bromoacetaldehyde at pH 6.5 and 37° for 15 min, 70% of the adenine residues was converted to 1,N^6-ethenoadenine.

Chloroacetaldehyde modification of adenine at the polynucleotide level has been shown to be single-strand specific.[4] The bromoacetaldehyde modification was also single-strand specific. As illustrated in Fig. 2, the adenine residues in native calf thymus DNA were unreactive, whereas those in denatured DNA reacted readily.

Reaction of Cytosine Derivatives with Bromoacetaldehyde

Reactivity of bromoacetaldehyde with the cytosine nucleus was also high. As shown in Fig. 3, the reaction between cytidine and bromoacetaldehyde proceeded quite readily in the pH range of 4.5—7.4, and the pH optimum was around 6. At pH 4.5, the rate of this reaction was about the same as that of adenylic acid modification. In the pH range of 5 to 8, the rates were greater than those of adenylate modification (compare Fig. 3 with Fig. 1). The reactivity difference between bromo- and chloroacetaldehyde was about 10-fold, as shown in the inset of Fig. 3.

Crystalline 3,N^4-ethenocytosine was obtainable in a good yield of 73% by incubating cytosine with bromoacetaldehyde at 37° and pH 6 for three days. It has been reported that this compound can be prepared in a yield of 25% by treatment of cytosine with chloroacetaldehyde for six weeks.[14]

In the reaction of cytidine with bromoacetaldehyde, the rate-limiting step for the overall conversion to 3,N^4-ethenocytidine was the second step, *i.e.* the dehydration of the intermediate II. The intermediate observable on the paper chromatogram was eluted and its properties were investigated. An attempt to isolate II in a pure state failed because a small amount of 3,N^4-ethenocytidine, formed from II during the work-up, contaminated the material. Other workers have also reported that the isolation of compound II from the reaction mixture of cytidine and chloroacetaldehyde was difficult because of its lability.[12,13] Compound II showed ultraviolet-absorption maxima at 285 nm in 0.1 N HCl, 280 nm in 0.2 M sodium phosphate, pH 7, and 278 nm in 0.1 N NaOH. The minima were at 244 nm in 0.1 N HCl, 247 nm in the phosphate buffer, and 250 nm in 0.1 N NaOH. These values are consistent with the ultraviolet absorption curves reported in the literature[13] for a sample of compound II. Complete conversion of II into 3,N^4-ethenocytidine required incubation for 48 hr at pH 5, or for 168 hr at pH 7, both at 37°. When heated at 80°, the conversion at pH 7 reached completion in 3 hr. The reaction of cytosine residues at the polynucleotide level was single-strand specific. Thus, poly(C) treated with 0.12 M bromoacetaldehyde at pH 7 and 37° for 90 min was modified to the extent of 90%, while no reaction was observed with poly(I)-poly(C) upon similar treatment.

Reaction of Guanosine 5′-Phosphate with Bromoacetaldehyde

The reaction of guanosine 5′-phosphate with bromoacetaldehyde at pH 7 and 37° was followed spectrophotometrically. Figure 4 shows the spectral change as a function of time of treatment. The reaction was close to completion by 7 hr. The spectrum showed a strong absorption maximum at 229 nm, suggesting the formation of 1,N^2-ethenoguanosine phosphate.[15] Analysis of the reaction product by paper chromatography (solvent; isobutyric acid–NH_3–H_2O, 75: 1: 24), however, indicated that the reaction yielded a complex mixture of products. A detailed study of the products will be the subject of a forthcoming paper. Although the reactivity of the guanine moiety towards bromoacetaldehyde was significant, the rate was considerably smaller than those of the adenine and cytosine modifications under similar conditions. At pH 5 the reaction was very slow; on treatment for 4 hr with 0.12 M bromoacetaldehyde, only 10% of guanosine 5′-phosphate was modified.

14) J.R. Barrio, P.D. Sattsangi, B.A. Gruber, L.G. Dammann, and N.J. Leonard, *J. Am. Chem. Soc.*, **98**, 7408 (1976).
15) P.D. Sattsangi, N.J. Leonard, and C.R. Frihart, *J. Org. Chem.*, **42**, 3292 (1977).

Table II. Mutagenicity of Bromo- and Chloroacetaldehyde as tested on *Salmonella typhimurium* TA 100

Reagent	Concentration of reagent (M)	Time of incubation (min)[a]	His$^+$ revertants per plate
Bromoacetaldehyde	5×10^{-5}	15	151
	5×10^{-5}	60	144
	5×10^{-4}	15	199
	5×10^{-4}	60	220
	5×10^{-3}	15	Killing
	5×10^{-3}	60	Killing
Chloroacetaldehyde	5×10^{-5}	15	160
	5×10^{-5}	60	197
	5×10^{-4}	15	286
	5×10^{-4}	60	506
	5×10^{-3}	15	995
	5×10^{-3}	60	1235
None		15	133

a) Bacteria were incubated with the reagent, before plating, for the time indicated. The incubation was at pH 7.4 and 37°.

Mutagenicity of Bromoacetaldehyde

Chloroacetaldehyde has been reported to be mutagenic towards *Salmonella typhimurium* TA 100.[16,17] Mutagenicity testing of bromoacetaldehyde (Table II) has shown that its mutagenic activity is very weak, if any, but it is rather toxic to the bacteria. Chloroacetaldehyde, on the other hand, did show significant mutagenicity at similar concentrations.

Discussion

The pH-dependence of the bromoacetaldehyde modification of adenine and cytosine derivatives is similar to that of chloroacetaldehyde reported in the literature.[4,12,13] Guanosine has been shown to react with chloroacetaldehyde at neutral pH but not at acidic pH[12,15] and this is also the case for bromoacetaldehyde. We have checked the reactivity of bromoacetaldehyde towards thymidine and uridine, and could detect no reaction upon incubation at 37° with 0.12 M bromoacetaldehyde at pH 7 or pH 9 for 2 hr (data not shown). The single-strand specific nature of the bromoacetaldehyde modification is the same as that with chloroacetaldehyde.[4]

The method for preparting bromoacetaldehyde is straightforward and posed no problem in repeated experiments. Better quality material was obtainable by Method 1 than by Method 2 (described in "Experimental Procedures"), but the reagent prepared by the simple method, Method 2, can also be used for the modification reactions.

Thus, bromoacetaldehyde can be used in place of chloroacetaldehyde in nucleic acid studies, offering the advantage of much faster modification than the latter reagent.

Handling of chloroacetaldehyde may be dangerous owing to the mutagenicity of the reagent.[15] Although bromoacetaldehyde might be expected to show stronger mutagenicity than chloroacetaldehyde, our present data (Table II) indicate that bromoacetaldehyde is only very weakly mutagenic, if at all, but is rather toxic to the bacteria.

Acknowledgement We thank Dr. M. Yoshioka of the Faculty of Pharmaceutical Sciences, University of Tokyo, for his advice on preparing bromoacetaldehyde.

16) J. McCann, V. Simmon, D. Streitwieser, and B. Ames, *Proc. Natl. Acad. Sci. USA*, **72**, 3190 (1975).
17) J.D. Elmore, J.L. Wong, A.D. Laumbach, and U.N. Streips, *Biochim. Biophys. Acta*, **442**, 405 (1976).