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Chloroacetaldehyde-Treated Ribo- and Deoxyribopolynucleotides. 1. Reaction Products†

J. T. Kuśmierek‡ and B. Singer*

ABSTRACT: The in vitro reaction of the vinyl chloride metabolite chloroacetaldehyde (CAA) with cytosine and adenine residues in ribo- and deoxyribopolynucleotides leads to the formation of the relatively stable hydrated etheno derivatives 3,N^4-(N^4-α-hydroxyethene)cytosine (εC·H_2O) and 1,N^6-(N^6-α-hydroxyethene)adenine (εA·H_2O). Under physiological conditions the hydrates are slowly converted to 3,N^4-ethenocytosine (εC) and 1,N^6-ethenoadenine (εA). The half-life at pH 7.25 of εC·H_2O in poly(rC) is 4.9 h at 50 °C and of εA·H_2O in poly(rA) is 1.4 h at 37 °C. These dehydration rates in polymers are similar to those for hydrates in monomers. The reactivity of A and C residues is greatly suppressed in double-stranded polymers. Adenine residues are about 10 times less reactive in poly(rA)·poly(rU) than A in single-stranded polymers. Under similar reaction conditions no reaction of C residues in poly(rC)·poly(rG) was detected. In vinyl chloride exposed cells, where CAA is formed, the cyclic etheno derivatives of A and C are likely to occur preferentially in single-stranded regions of nucleic acids, with the hydrate forming a major proportion of the modification.

Chloroacetaldehyde (CAA)[1] has long been used to prepare fluorescent etheno derivatives of cytidine and adenosine. Although the reaction mechanism postulates two intermediates, these are unstable, particularly in acid. Most previous workers used pH 3–5 for modification reactions and thus obtained only the etheno compounds (Kochetkov et al., 1971; Barrio et al., 1972; Secrist et al., 1972). Later Wiewiórowski and coworkers (Biernat et al., 1978; Krzyżosiak et al., 1979) did isolate the hydrated intermediates and determined, at pH 3–7, the conditions and rates for dehydration leading to etheno derivatives in monomers. These rates of dehydration were much slower under physiological conditions than those in more acid solution. Although Krzyżosiak et al. (1981) had indications that the hydrates were formed in CAA-treated tRNA, they did not isolate these compounds but studied their stability in the RNA. Nevertheless, investigators have reacted polynucleotides with CAA or cells with vinyl chloride, where CAA is formed, and have assumed that only the etheno derivatives would be present. We now report that after reaction of CAA with poly(rC), poly(dC), poly(rA), and poly(dA) at neutrality, the hydrate is the predominant product and is only converted to the etheno derivative when heated several hours at elevated temperatures. It would thus appear that when CAA-treated polynucleotides are directly used for transcription studies, both hydrate and dehydrated etheno compounds can be present. Therefore, transcription results with CAA-modified polymers are likely to represent data for both types of compounds rather than for only the etheno compounds (Barbin et al., 1981; Hall et al., 1981). In the succeeding paper (Kuśmierek & Singer, 1982), we report the effect on transcription of each derivative separately.

Materials and Methods

Materials. Chloroacetaldehyde (CAA), 45% in water solution, was an ICN Pharmaceuticals product. 3,N^4-Ethenocytidine 5′-diphosphate and 1,N^6-ethenoadenosine 5′-di-

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* Address correspondence to this author at the Laboratory of Chemical Biodynamics, University of California, Berkeley, CA 94720.
‡ Permanent address: Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw 02532, Poland.

[1] Abbreviations: HPLC, high-pressure liquid chromatography; Tris, tris(hydroxymethyl)aminomethane; CAA, chloroacetaldehyde; εCyd, 3,N^4-ethenocytidine (5,6-dihydro-5-oxo-6-β-D-ribofuranosylimidazo[1,2-*c*]pyrimidine); εCyd·H_2O, hydrated, 3,N^4-ethenocytidine, 3,N^4-(N^4-α-hydroxyethane)cytidine (2-hydroxy-2,3,5,6-tetrahydro-5-oxo-6-β-D-ribofuranosylimidazo[1,2-*c*]pyrimidine); εAdo, 1,N^6-ethenoadenosine (3-β-D-ribofuranosylimidazo[2,1-*i*]purine); εAdo·H_2O, hydrated 1,N^6-ethenoadenosine, 1,N^6-(N^6-α-hydroxyethene)adenosine (7,8-dihydro-8-hydroxy-3-β-D-ribofuranosylimidazo[2,1-*i*]purine); εC, εC·H_2O, εA, and εA·H_2O stand for modified base residues in polymers. The use of H_2O in the abbreviations represents the hydrated form of εA and εC, rather than water of hydration.

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phosphate were from P-L Biochemicals. After dephosphorylation by bacterial alkaline phosphatase the resulting nucleosides were used as authentic 3,N^4-ethenocytidine and 1,N^6-ethenoadenosine. All ribo- and deoxyribopolynucleotides were purchased from Miles and P-L Biochemicals. Both snake venom phosphodiesterase and bacterial alkaline phosphatase were from Worthington.

Preparation of Poly(rG)·Poly(rC) Complex. The complex poly(rG)·poly(rC) was prepared according to Golaš et al. (1980) and isolated by precipitation with ethanol from tetraethylammonium chloride solution. Poly(rG)·poly(rC) tends to form gels at high concentrations. However, it is possible to prepare a solution containing about 60 OD_{max}/mL in 0.1 M pH 7.25 sodium cacodylate by keeping the mixture for several hours at 37 °C. The UV spectrum of this complex in water shows a sharp peak of absorbancy with λ_{max} = 258 nm, whereas the UV spectrum of the equimolar mixture of poly(rG) and poly(rC) shows a broad peak with λ_{max} = 255 nm and a shoulder at about 275 nm. The complex when heated in tetraethylammonium chloride solutions exhibits sharp temperature profiles with T_m values and hyperchromicity depending on the salt concentrations (data not shown).

Reactions of Polynucleotides with Chloroacetaldehyde (CAA). Polymers were reacted at 37 °C in 0.1 M sodium cacodylate buffers (pH 4.0–8.1) with a 45% aqueous solution of CAA for various times. The reaction was terminated by addition of an equal volume of 3 M pH 5 sodium acetate and precipitation of reacted polymer by addition of 3–5 volumes of absolute ethanol. After centrifugation at 0 °C the polymer pellet was redissolved in 0.1–0.2 mL of 0.1 M pH 7.25 sodium cacodylate and twice reprecipitated with absolute ethanol. After being washed twice with absolute ethanol and once with ether (centrifugation after each washing), the reacted polymer was dried at room temperature for several minutes. Reacted polymers were dissolved in 0.01 M pH 7.25 Tris-HCl buffer.

In order to achieve the complete conversion of hydrated etheno derivatives to etheno derivatives, we heated part of each polymer sample in 0.01 M pH 7.25 Tris-HCl at 85–90 °C. Adenine-containing polymers were heated 1 h, and cytosine-containing polymers were heated 2 h. All polymer solutions were stored frozen at −70 °C.

Hydrolysis of CAA-Modified Polymers. The conditions used for enzyme digestion to nucleosides were as follows: approximately 1 A_{260} unit of polymer in 0.1 mL of 0.01 M pH 7.25 Tris-HCl buffer was incubated for 18 h at 37 °C with 5 μg each of snake venom phosphodiesterase and bacterial alkaline phosphatase. In order to detect hydrated etheno derivatives, we digested the nonheated polymers using twice the amount of enzymes and a shortened digestion time of 4–10 h.

Guanosine-containing polymers were poorly digested under the standard conditions. In this case, after 18-h digestion the same enzymes were again added, and the incubation was continued an additional 24 h. Polymers were also hydrolyzed with 1 M HCl for 1 h at 100 °C.

HPLC Analysis of CAA-Modified Polymers. Samples of hydrolyzed polymers were analyzed on a 250 × 4 mm Aminex HPLC cation-exchange column (NH_4^+ form) (Bio-Rad) with 0.4 M pH 7 ammonium formate as the solvent. The eluate was recorded at 254 nm. The column was routinely operated at 55 °C, but in some cases the column was operated at room temperature (20 °C) in order to detect ε$Cyd·H_2O$ and ε$Ado·H_2O$. The flow at 55 °C was 0.45 mL/min, whereas at 20 °C it was 0.36 mL/min. For qualitative identification of the products, the peaks from HPLC were collected, and UV spectra of eluates were recorded. Quantitative analysis was done by gravimetric integration of the peaks. Molar extinction coefficients at λ = 254 (Cyd, 6.6 × 10^3; εCyd, 6.9 × 10^3; Ado, 13.7 × 10^3; εAdo, 4.7 × 10^3) were calculated from UV spectra recorded in 0.4 M pH 7 ammonium formate. Original extinction coefficients (at λ_{max}) of εCyd and εAdo were taken from Barrio et al. (1972). The values of extinction coefficients for deoxyribonucleosides were assumed to be the same as for corresponding ribonucleosides. The retention times of nucleosides are in Table I.

Table I: HPLC Retention Times of Ribo- and Deoxyribonucleosides and Their CAA Modified Derivatives[a]

compound	retention time (min) at 55 °C[b]	retention time (min) at 20 °C[c]
cytidine	5.3	9.4
3,N^4-ethenocytidine	7.8	17.2
3,N^4-(N^4-α-hydroxyethene)cytidine	28.0	40.0
adenosine	7.4	17.4
1,N^6-ethenoadenosine	13.3	34.4
1,N^6-(N^6-α-hydroxyethene)adenosine		100.0
guanosine	9.0	
uridine	3.8	
deoxycytidine	6.5	9.9
3,N^4-ethenodeoxycytidine	10.8	23.0
3,N^4-(N^4-α-hydroxyethene)deoxycytidine		42.0
deoxyadenosine	9.9	22.5
1,N^6-ethenodeoxyadenosine	20.1	53.0
1,N^6-(N^6-α-hydroxyethene)deoxyadenosine		115.0

[a] See Materials and Methods for HPLC conditions. Figures 3 and 4 illustrate some separations. [b] The flow was 0.45 mL/min at 55 °C. [c] The flow was 0.36 mL/min at 20 °C.

Determination of Half-Life of ε$C·H_2O$ and ε$A·H_2O$ in Poly(rC) and Poly(rA). The half-lives were determined spectrophotometrically by using a Varian-Cary 219 spectrophotometer equipped with a thermostated cell chamber. The absorption changes accompanying the dehydration reaction were monitored at 230 and 258 nm in all pH ranges studied with modified poly(rA). The changes accompanying dehydration of ε$C·H_2O$ in poly(rC) were monitored at 230, 280, and 300 nm in the pH range 4.6–6.0 and at 230, 270, and 300 nm in the pH range 7.25–8.1. In all the cases there was a linear dependence between the logarithm of relative change and time, indicating first-order kinetics. Dehydration of ε$Cyd·H_2O$ and ε$Ado·H_2O$ was studied only at pH 7.

Results

Reaction of Cytosine and Adenine Residues in Polymers with CAA. 3,N^4-Ethenocytidine (εCyd) and 1,N^6-ethenoadenosine (εAdo), as well as their hydrated intermediates (ε$Cyd·H_2O$ and ε$Ado·H_2O$), were found as the sole products of reaction of CAA with poly(rC) and poly(rA), respectively, after enzymatic digestion of polymers reacted to 50% modification. Only traces of other unidentified products were found in polymers reacted almost to completion. Both εCyd and εAdo from polymers were identified by their UV spectra (Barrio et al., 1972) (Figures 1 and 2) and HPLC retention times (peak II in Figures 3 and 4 and Table I).

Hydrated etheno derivatives are unstable as monomers and can only be detected with mild methods for analysis of reacted polymers. Decreasing the time of enzymatic digestion and performing HPLC analysis at room temperature were necessary to obtain ε$Cyd·H_2O$ and ε$Ado·H_2O$ as separate peaks (Figures 3 and 4) in order to study their physicochemical properties. The hydrated derivatives, ε$Cyd·H_2O$ and ε$Ado·H_2O$, were identified by comparison of their UV spectra with

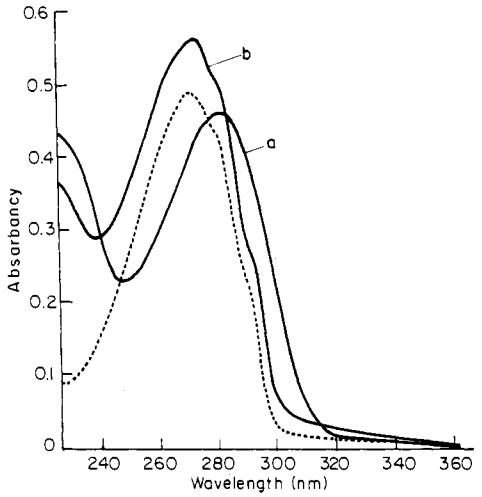

FIGURE 1: UV spectra of nucleosides isolated from HPLC analysis of CAA-treated poly(rC): (a) ϵCyd·H_2O; (b) ϵCyd·H_2O after heating 0.5 h at 85 °C; (···) ϵCyd. All spectra were recorded in 0.4 M pH 7 ammonium formate. ϵCyd and ϵCyd·H_2O are peaks II and III in Figure 3.

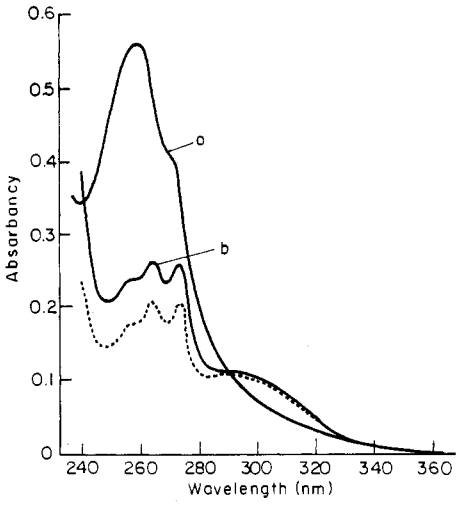

FIGURE 2: UV spectra of nucleosides isolated from HPLC analysis of CAA-treated poly(rA): (a) ϵAdo·H_2O; (b) ϵAdo·H_2O after heating 3.5 h at 37 °C; (···) ϵAdo. All spectra were recorded in 0.4 M pH 7 ammonium formate. ϵAdo and ϵAdo·H_2O are peaks II and III in Figure 4.

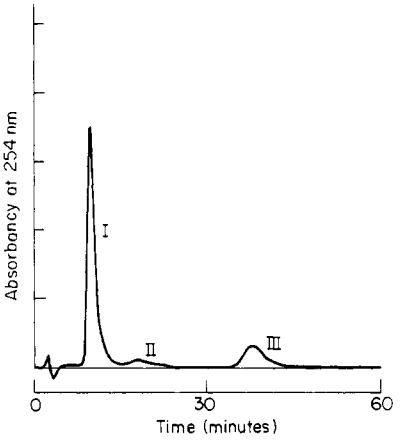

FIGURE 3: HPLC separation at 20 °C of products of enzymatic digestion of CAA-treated poly(rC). Peak I is Cyd, peak II is ϵCyd, and peak III is ϵCyd·H_2O. The total modification was 36%, and enzyme digestion was for 10 h.

the published spectra (Krzyżosiak et al., 1979) and by the spectrally monitored heat conversion of these derivatives into

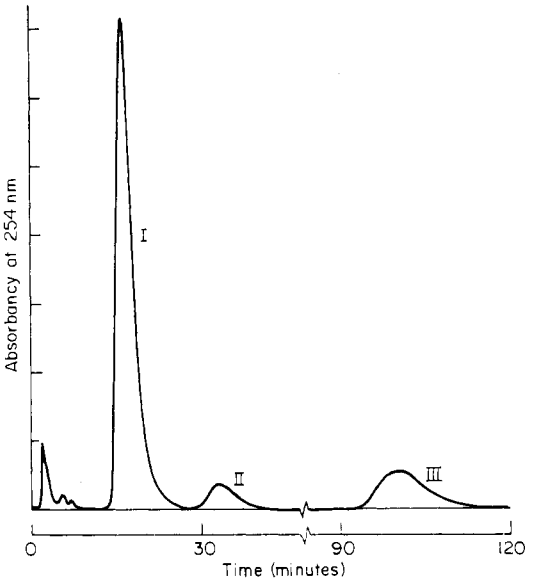

FIGURE 4: HPLC separation at 20 °C of products of enzymatic digestion of CAA-treated poly(rA). Peak I is Ado, peak II is ϵAdo, and peak III is ϵAdo·H_2O. The total modification was 36%, and enzyme digestion was for 4 h.

Table II: Time Course of Reaction of Poly(rC), Poly(dC), Poly(rA), and Poly(dA) with Chloroacetaldehyde at Neutrality [a]

time of reaction (h)	extent of modification (%) in			
	poly(rC)	poly(dC)	poly(rA)	poly(dA)
0.25	9	8	3	
0.5	16	13	7	2
1.0	21	24	11	4
2.0	36		23	9
4.0			36	
8.0	78		55	
16.0	93		81	

[a] The polymers were reacted in 0.1 M sodium cacodylate buffer, pH 7.25, at 37 °C. The concentration of polymers was as follows: poly(rC), 90 OD_{max}/mL; poly(dC), 70 OD_{max}/mL; poly(rA), 110 OD_{max}/mL; poly(dA), 90 OD_{max}/mL. CAA (45% solution in water) was added in the proportion 0.025 mL to 1 mL of polymer solution in each case. The pH of the reaction mixture (determined in a separate sample containing buffer and reagent only) decreased during the course of reaction from 7.25 at zero time, to 7.0 after 2 h, to 6.9 after 4 h, and to 6.4 after 24 h.

ϵCyd and ϵAdo (Figures 1 and 2). The hydrated derivatives after conversion show the same retention times on HPLC as authentic ϵCyd and ϵAdo. Another indication supporting the identity of hydrated derivatives is that they have longer retention times on the cation-exchange column used compared to the retention time of ϵCyd and ϵAdo. This would be expected since hydrated derivatives exhibit higher pK_a values than dehydrated ones. The pK_as for ϵCyd·H_2O and ϵAdo·H_2O are 6.9 and 8.1 (Krzyżosiak et al., 1979) whereas the pK_as for ϵCyd and ϵAdo are 3.7 and 3.9 (Secrist et al., 1972).

The reaction products of poly(dC) and poly(dA) were not studied in as great detail. Both the hydrated and dehydrated products of CAA reaction were identified on the basis of analogy to the known products derived from the corresponding ribopolymers.

The time course of reaction of poly(rC), poly(dC), poly(rA), and poly(dA) is shown in Table II. Under comparable conditions at neutrality, as well as at lower pH, cytosine-containing polymers react faster than adenine-containing polymers. The higher reactivity of cytidine in comparison to adenosine was also found in the reaction of these monomers with CAA in

Table III: pH Dependence of Extent of Modification of Poly(rC) and Poly(rA) with Chloroacetaldehyde[a]

pH[b]	extent of modification (%) in poly(rC)	poly(rA)
8.1	60	33
7.25	63	31
6.0	66	38
5.0	9	2[c]
4.0	6[c]	2[c]

[a] The polymers were reacted in 0.1 M sodium cacodylate buffers for 2 h at 37 °C. The concentration of polymers was as follows: poly(rC), 150 OD_{max}/mL; poly(rA), 160 OD_{max}/mL. CAA (45% solution in water) was added in the proportion 0.05 mL to 1 mL of polymer solution in each case. [b] The final pHs in controls lacking polymer were 6.9, 6.8, 5.9, 4.7, and 3.1. [c] Precipitation of polymer occurred.

Table IV: Reactivity of C and A Residues in Double-Stranded and Single-Stranded Ribopolymers Reacted with Chloroacetaldehyde[a]

polymer	polymer concn (A_{max}/mL)	modification of C or A[b] (%)
poly(rC)·poly(rG)	60	0
poly(rG) plus poly(rC)	50	54
poly(rC)	30	70
poly(rG)	40	
poly(rG,rU) (~1:1)	140	
poly(rG,rA) (~1:1)	160	61
poly(rA)·poly(rU)	130	6
poly(rA)	80	48
poly(rU)	130	

[a] Polymers in 0.2 mL of 0.1 M pH 7.25 sodium cacodylate buffer were reacted with 0.05 mL of 45% aqueous solution of CAA at 37 °C, 1 h. [b] No reaction was detected with U or G residues. See Materials and Methods for analytical methods.

the pH range 4–7 (Biernat et al., 1978). The reactivity of poly(rC) is similar to the reactivity of poly(dC), whereas poly(rA) is much more reactive than poly(dA) (Table II).

The overall modification of C bases in poly(rC) slightly increases when the pH of the reaction decreases from 8.1 to 6.0 (Table III). Further decrease of pH causes a dramatic decrease in the extent of modification. At pH 5 only 9% of C residues are modified. At pH 4 the precipitation of poly(rC) occurs during the course of reaction. Nevertheless 6% of the C residues were found modified. The same trend is observed in the case of poly(rA) (Table III). At pH 5 and pH 4 poly(rA) precipitates from solution, and only 2% of the A residues were found modified in both cases.

In view of the fact that the reactivity of monomeric cytidine is almost the same in the pH range 5–7 (Biernat et al., 1978), the great decrease in reactivity of poly(rC) below pH 6 can be attributed to the formation of the protonated secondary structure of poly(rC). Unfortunately, because of the precipitation of polymer at pH 5 and below, a similar study with poly(rA) has not been done.

The influence of secondary structure on the reactivity of C and A residues was studied by using poly(rC)·poly(rG) and poly(rA)·poly(rU) as the double-stranded polymers (Table IV). The C residues were found unreactive in the poly(rC)·poly(rG) complex whereas under comparable reaction conditions poly(rC) in a mixture with poly(rG) or poly(rC) alone reacts to a high extent (Table IV). The reactivity of A residues in the poly(rA)·poly(rU) complex was about 10 times less than in single-stranded poly(rA) or in poly(rA,rG). The difference between the reactivity of poly(rC)·poly(rG) and poly(rA)·poly(rU) can be attributed to the much lower stability of the latter complex in comparison to the former.

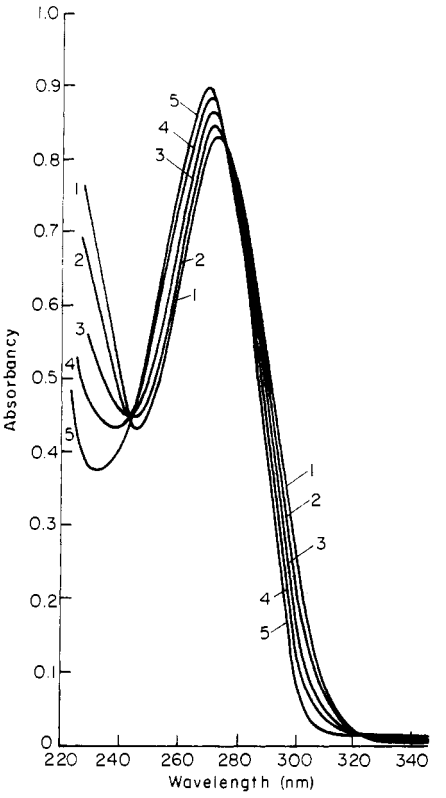

FIGURE 5: Spectral changes accompanying dehydration of $\epsilon C \cdot H_2O$ residues in poly(rC) at 50 °C: (1) 0 time, (2) 2 h, (3) 5 h, (4) 10 h and (5) 29 and 35 h. The polymer was heated in 0.1 M sodium cacodylate buffer, pH 7.25.

Table V: Half-Life of the Conversion of Hydrated Etheno Derivatives into Etheno Derivatives in Poly(rA) and in Poly(rC)[a]

polymer	temp	half-life (h) at pH[b] 4.0	4.6	5.0	6.0	7.25	8.1
poly(rA), 28% modified[c]	37 °C	1.1		1.1	0.9	1.4	2.9
poly(rC), 63% modified[d]	50 °C		1.8[e]	1.8	1.7	4.9	14.3

[a] The method for determination of half-life is described under Materials and Methods. [b] All buffers were 0.1 M sodium cacodylate. [c] Half-life of monomeric $\epsilon Ado \cdot H_2O$ in 0.4 M pH 7 ammonium formate at 37 °C is 1.7 h. [d] Half-life of monomeric $\epsilon Cyd \cdot H_2O$ in 0.4 M pH 7 ammonium formate at 50 °C is 5.0 h. [e] Half-life measured at 37 °C is 15.4 h.

Uracil and guanine residues were found unreactive in both single- and double-stranded polymers. CAA-treated polymers containing G were hydrolyzed enzymatically to give G nucleosides and with 1 M HCl to give G bases. No products other than guanosine or guanine were found upon HPLC separation. This is somewhat surprising in view of the fact that guanosine itself reacts with CAA to a high extent under comparable conditions, giving a complex mixture of products. Although these products have not been identified, none were found in CAA-reacted poly(rG,rU), poly(rG,rA), poly(rG), or poly(rC)·poly(rG).

Dehydration of $\epsilon C \cdot H_2O$ and $\epsilon A \cdot H_2O$ Residues in Poly(rC) and Poly(rA). The dehydration reaction was studied spectrophotometrically, exploiting the changes of UV spectra accompanying the conversion of hydrated etheno derivatives into etheno derivatives (Figures 5 and 6). The half-lives in the pH range 4–8.1 (using the average of values obtained for different wavelengths) of $\epsilon C \cdot H_2O$ residues in 63% modified poly(rC) at 50 °C and $\epsilon A \cdot H_2O$ residues in 28% modified

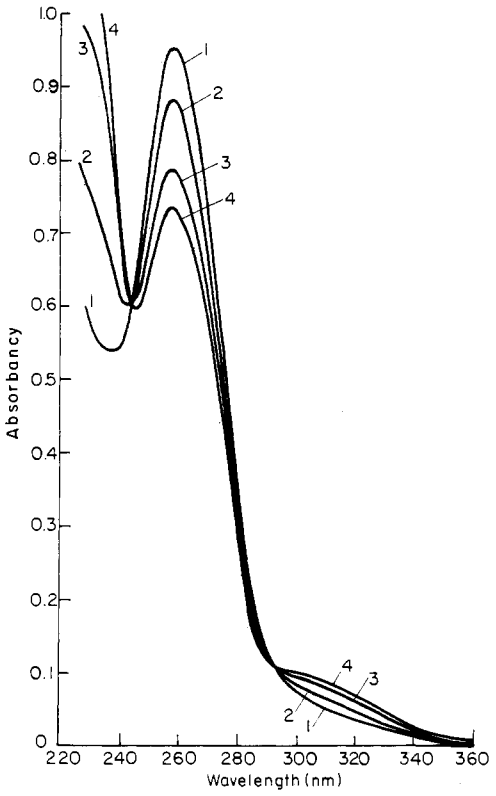

FIGURE 6: Spectral changes accompanying dehydration of $\epsilon A \cdot H_2O$ residues in poly(rA) at 37 °C: (1) 0 time, (2) 1 h, (3) 3 h, and (4) 7 and 9 h. The polymer was heated in 0.1 M sodium cacodylate buffer, pH 7.25.

poly(rA) at 37 °C are given in Table V. The temperature of 50 °C for dehydration of modified poly(rC) was chosen since at 37 °C the changes were too slow to be measured in a reasonable time. A comparison, at pH 7, of the dehydration rates of hydrates in monomers and polymers shows that the polymeric structure does not affect the rate (Table V).

Discussion

The mechanism of reaction of CAA with cytidine and adenosine postulated recently by Kyzyżosiak et al. (1979) is presented in Figure 7 where only the reactive part of both nucleosides is shown. The only stable intermediates that have been isolated and characterized are intermediates **3**.

The products of reaction of an aldehyde group with exocyclic amino groups of Cyd or Ado were postulated to be intermediates **2** on the basis that during attempted isolation they convert spontaneously to the parent nucleosides, **1**. If, alternatively, the primary products of reaction were alkylated on the endocyclic nitrogen atom (structure not shown), they would be converted to **3** but not to **1**.

The reaction of CAA with poly(rC), poly(dC), poly(rA), and poly(dA) leads also to formation of hydroxyethane derivatives **3** at the polymer level, which has been shown by isolation of these derivatives after enzymatic digestion of reacted polymers (Figures 1 and 2, Table I). Another indication that hydroxyethane derivatives are present in reacted polymers is that the UV spectra of reacted polymers undergo changes during heating (Figures 5 and 6), and the digestion of heated polymers leads to isolation only of the parent nucleosides, **1**, and stable etheno derivatives, **4**.

The dehydration rates are pH dependent. $\epsilon C \cdot H_2O$ residues in poly(rC) are dehydrated at about the same rate over the pH range 4.6–6.0. This reaction is considerably slower at higher pHs. The optimum for dehydration of $\epsilon A \cdot H_2O$ in poly(rA) is shifted about 1 pH unit toward higher pHs. Similar relative pH dependence is also observed for monomers (Krzyżosiak et al., 1979). This implies that only the protonated species undergo dehydration since the pK_a of protonation of $\epsilon Cyd \cdot H_2O$ is 6.9 and of $\epsilon Ado \cdot H_2O$ is 8.1. Considering that most chemical reactions are slower in polymers, it is interesting that the dehydration rates of both $\epsilon C \cdot H_2O$ and $\epsilon A \cdot H_2O$ are almost the same in monomers and polymers (Table V).

Cytosine residues are more reactive than adenine residues in polymers over the pH range studied (Tables II and III). This preference for reaction of Cyd residues was also found for monomers with both chloroacetaldehyde (Biernat et al., 1978) and bromoacetaldehyde (Kayasuga-Mikado et al., 1980). The reactivity of A and C residues is greatly suppressed in double-stranded polymers (Table IV). Adenine residues are about 10 times less reactive in poly(rA)·poly(rU) than in A in single-stranded polymers. Under similar reaction conditions no modification of C residues in poly(rC)·poly(rG) is found although in single-stranded polymers C is more reactive than A.

When animals were exposed to [^{14}C]vinyl chloride by inhalation, ϵC and ϵA were detected in liver RNA but not in DNA (Laib et al., 1981). However, in experiments in which rats were chronically exposed to low levels of unlabeled vinyl chloride in drinking water for 2 years, both ϵA and ϵC were found in DNA by mass spectrometry (Green & Hathway, 1978). It is not surprising that Laib et al. (1981) found ϵC and ϵA in RNA since it contains considerable amounts of single-stranded regions. Neither would it be expected that a short exposure by inhalation would produce measurable amounts of etheno derivative in DNA. Long exposure, as shown by Green & Hathway (1978), increases the probability of modification due to local denaturation and replication of the DNA.

Our search for reaction products of CAA with G residues in polynucleotides (Table IV) was done for two reasons. One was that guanosine was found reactive with CAA in the pH range 5–7, although to much lower extent than cytidine or adenosine (Sattsangi et al., 1977; Biernat et al., 1978). The main product isolated was identified as 1,N^2-ethenoguanosine (Sattsangi et al., 1977). Another reason for our study was that 7-(oxoethyl)guanine was found in DNA isolated from vinyl chloride treated animals (Osterman-Golkar et al., 1977;

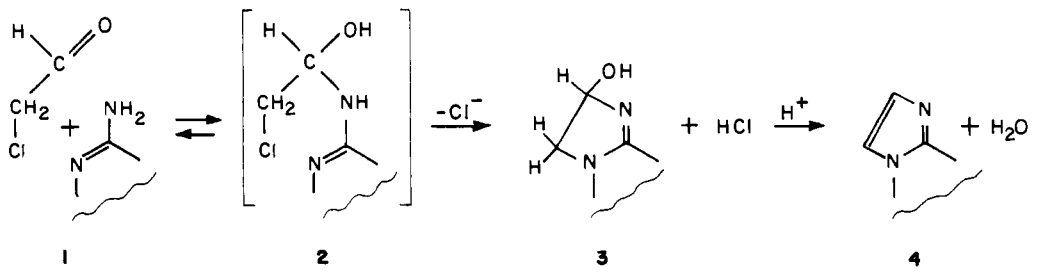

FIGURE 7: Postulated mechanism for reaction of chloroacetaldehyde with cytidine and adenosine.

Laib et al., 1981), as well as in vitro experiments where DNA was incubated with vinyl chloride in the presence of rat liver microsomes (Laib et al., 1981). In both cases vinyl chloride is metabolically converted to two active species, chloroethylene oxide and chloroacetaldehyde (Barbin et al., 1975).

At pH 7, G residues do not form detectable amounts of reaction products in polymers, in contrast to monomers, under conditions where A and C residues in polymers react to a high extent. It seems that the formation of 7-(oxoethyl)guanine residues in DNA reacted with metabolically activated vinyl chloride (Osterman-Golkar et al., 1977; Laib et al., 1981) is due to reaction with one metabolite, chloroethylene oxide, but not with the other metabolite, chloroacetaldehyde. In vitro, 7-(oxoethyl)deoxyguanosine has been formed by reacting chloroethylene oxide with deoxyguanosine in glacial acetic acid (Scherer et al., 1981). Chloroethylene oxide is similar in reactivity to other aliphatic epoxides. Thus alkylation by this metabolite of sites other than the N-7 of G in DNA can be expected (Lawley & Jarman, 1972; Hemminki et al., 1980).

Our conclusion, based on results from this laboratory and from other investigators, indicates that two of the metabolites of vinyl chloride, chloroethylene oxide and chloroacetaldehyde, react with nucleic acids according to two different mechanisms. Chloroethylene oxide, like other aliphatic epoxides, attacks DNA at N-7 of G (and probably at other sites). Chloroacetaldehyde reacts with C and A residues in single-stranded regions, leading to the formation of hydrated etheno derivatives that are subsequently converted to ethano derivatives.

At this time there are no reports regarding the repair of vinyl chloride adducts in vivo. N^7-(Oxoethyl)guanine is probably released spontaneously, like other 7-alkylguanines, giving apurinic sites that can be repaired in the usual way. Etheno derivatives of C and A are chemically stable under physiological conditions. The glycosidic bond in 5′-dϵAMP is more stable than in 5′-dAMP during HCl hydrolysis (Kochetkov et al., 1973). The spontaneous release of ϵA residues from DNA, reported by Green & Hathway (1978), has not been confirmed by Hall et al. (1981). Our results also indicate that ϵC and ϵA are not released from chloroacetaldehyde-treated poly(dC) and poly(dA) to a greater extent than unmodified C and A when heated at neutrality (following paper). It is very likely that repair of ϵC and ϵA is not very efficient since these derivatives can be detected, without the carcinogen having a radioactive label, in DNA of rats exposed chronically to vinyl chloride at a level of 250 ppm in drinking water (Green & Hathway, 1978).

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