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Smooth and selective formation of the cyclic 1,N^2-propano adducts in the reactions of guanine nucleosides and nucleotides with acetaldehyde

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Abstract—The treatment of guanine nucleosides and nucleotides with excess acetaldehyde in pH 8.0 phosphate buffer containing a basic amino acid such as arginine and lysine resulted in the smooth and selective formation of the corresponding cyclic 1,N^2-propano adducts even under mild conditions.

Chemical modifications of nucleosides and nucleotides by acetaldehyde (AA) have been investigated in connection with the toxicity, mutagenicity, and carcinogenicity of AA commonly existing as exogenous and endogenous sources, such as the primary metabolite of ethanol, a component in tobacco smoke, and a metabolic intermediate of sugars, in the human environment.[1] From this viewpoint, it has been documented that the reactions of nucleic acids with AA under physiological conditions proceed chemo- and regio-selectively at the exocyclic amino group of the guanine moiety to form the easily hydrolyzable N^2-ethylidene guanines (**A**) as the major product, together with trace amounts of stable adducts such as N^2-(6-methyl-1,3-dioxan-4-yl)guanine adducts (**B**) and cyclic 1,N^2-propano guanine adducts (**C**).[2]

We now report that the reactions of guanine nucleosides and nucleotides with AA are significantly accelerated even under mild conditions by the presence of a basic amino acid such as arginine and lysine in the medium to give the corresponding cyclic 1,N^2-propano adducts (**1**) (cf., **C**: R = OH) almost quantitatively (Fig. 1).

The treatment of 2′-deoxyguanosine with excess AA in 0.1 M phosphate buffer (pH 8.0) at 37°C for 1 day in a sealed tube resulted in the formation of only a trace amount of the cyclic 1,N^2-propano adduct (**1a**) with recovery of most of the starting deoxyguanosine. Contrary to this fact, when the reaction was carried out in the presence of two equimolar amounts of arginine, the starting deoxyguanosine was smoothly consumed and almost quantitatively converted into **1a** as a 1:1 mixture of the diastereomers. Analogous results were obtained when using other amino acids such as lysine and cysteine in place of arginine, though their efficiencies for the cyclic adduct formation were dependent on the nature of the functional groups in the employed amino acids as shown in Table 1.

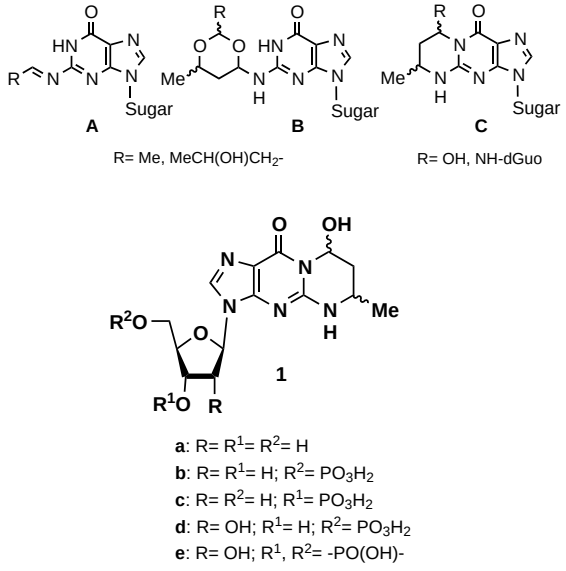

Figure 1.

Keywords: cyclic 1,N^2-propano guanine adduct; guanine nucleosides and nucleotides; acetaldehyde.

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Table 1. Reactions of 2′-deoxyguanosine with AA in the presence of amino acids

Amino acids	1,N^2-Cyclic adduct **1a** (recovered SM)%
L-Alanine	35 (58)
L-Serine	39 (57)
L-Tyrosine	26 (70)
L-Aspartic acid	3 (97)
L-Histidine	2 (98)
L-Cysteine	46 (54)
L-Lysine	59 (41)
L-Arginine	85 (15)

Reaction conditions: a mixture of 2′-deoxyguanosine (7.2 mg, 0.025 mmol) and an appropriate amino acid (0.05 mmol) in 0.1 M phosphate buffer (pH 8.0) (1.5 mL) containing AA (50 μL, 0.89 mmol) was heated at 37°C in a sealed tube for 8 h. The formation of the cyclic adduct **1a** and the recovery of the starting deoxyguanosine were estimated by TLC densitometric analysis [eluent: chloroform/methanol/acetic acid (40:8:1); detector: 254 nm].

The structure of the adduct **1a** was confirmed by comparison with the previously reported UV, mass, and ^{1}H NMR spectral data[2,3] [e.g. its ^{1}H NMR spectrum showed characteristic signals for the cyclic propano group at δ 1.16 (3H, d, $J=6$ Hz, NHCH*Me*; δ_C 20.6), 1.43 and 2.05 (each 1H, br t and br d, $J=$each 12 Hz, CHCH_2CHMe; δ_C 34.7), 3.63 (1H, m, NHC*H*Me; δ_C 41.3), and 6.03 and 6.07 (each 1/2H, each br s, NC*H*OH; δ_C 72.0) ppm] and by its $NaBH_4$-reduction to give N^2-(3-hydroxy-1-methylpropyl)-2′-deoxyguanosine.[4] The cyclic adduct **1a** was very stable in both acidic and basic medium; for example, even after heating overnight at 70°C in a pH 2 or pH 10 solution, the starting adduct was recovered unchanged. However, the adduct **1a** underwent acid hydrolysis at 90°C in the pH 2 solution leading to the deglycosylated product,[3] and its alkaline hydrolysis at 90°C in the pH 10 solution produced 2′-deoxyguanosine.

The formation of the cyclic adduct **1a** in the reaction of 2′-deoxyguanosine with AA in the presence of arginine was pH-dependent, and the employment of a basic buffer was effective for the adduct formation as shown in Fig. 2. When the reaction was carried out in a deuterated pH 8.0 buffer solution and followed by ^{1}H NMR spectroscopy, the smooth formation of 2-butenal or its equivalent (detected as 2-butenal after $CDCl_3$-extraction of the reaction mixtures) during the reaction was observed, together with the formation of 3-hydroxybutanal or its equivalent. The generation of 2-butenal (or its equivalent) in this reaction was parallel to the formation of the cyclic adduct **1a** under the conditions employed. The advanced formation of **1a** in the presence of arginine and the pH-dependency for the adduct formation were also observed in the reaction of 2′-deoxyguanosine with 2-butenal as shown in Fig. 2.

On the basis of the above facts and chemical reactivity of AA, 2-butenal, and the guanine moiety, the formation of the cyclic 1,N^2-propano adduct **1a** in the present reaction can be reasonably explained as illustrated in Scheme 1.

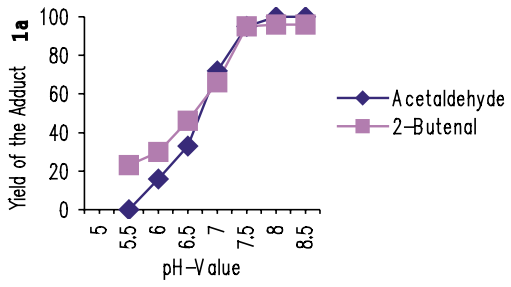
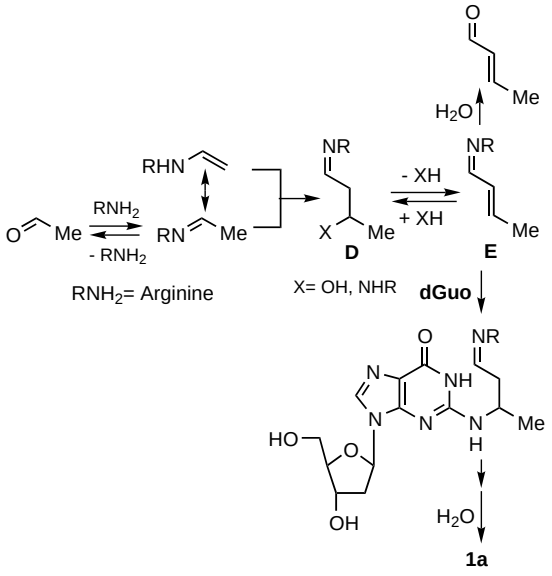


Figure 2. pH-Dependency of the reaction of 2′-deoxyguanosine with AA or 2-butenal in the presence of arginine.



Scheme 1.

The aldol condensation of AA to 3-hydroxybutanal (or its equivalent, **D**) followed by dehydration can be accelerated by the presence of arginine to form a Shiff base of 2-butenal, (**E**), as the key intermediate.[5] The Michael addition of the exocyclic amino group in 2′-deoxyguanosine to the 2-butenylidene moiety in **E** followed by ring-closure and the subsequent hydrolytic elimination of arginine affords the cyclic adduct **1a** as the ultimate product.

Analogous results were obtained in the reactions of guanine nucleotides such as 5′-dGMP, 5′-GMP, and 3′,5′-cyclic GMP with AA in the presence of arginine to give the corresponding cyclic 1,N^2-propano adducts **1b–e** in high yields, respectively.[6] It should be noted that no formation of any stable adducts was observed in the reactions of the adenine nucleosides and pyrimidine nucleosides under the same conditions (in the presence of arginine, pH 8.0 buffer, at 37°C, for 1 day), suggesting that the cyclic adduct formation with AA in the presence of the basic amino acids is also chemo- and regio-selective to the guanine nucleosides and nucleotides. Recently, the cyclic 1,N^2-propano adduct **1c** has been detected in the range of 2–3 adducts/10^9 nucleotides in the tissues of 2-butenal-treated and

untreated laboratory animals after enzymatic hydrolyses of the liver DNAs.[7] The results described above are interesting in connection with the molecular mechanisms explaining the toxic, mutagenic, and carcinogenic effects of AA,[1] and provide a convenient preparative method for the cyclic 1,N^2-propano adducts of the guanine nucleosides and nucleotides, **1a**–**e**.

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6. Isolated yields (in 0.1 mmol scale experiments) and spectral data of the cyclic adducts **1a**–**e** are as follows:
   Cyclic 1,N^2-propano dGuo adduct (1a):[3] 92% yield (as a 1:1 mixture of its diastereomers isolatable on HPLC); mp 155–160°C (from acetone); IR (KBr): 3394, 1686, 1571 cm^{-1}; UV (MeOH): 275 (sh), 261 nm; Mass (FAB^+): 338 $[M+H]^+$; HR-FAB MS: 338.1476 (mmu: +1.1; calcd for $C_{14}H_{20}N_5O_5$); ^1H NMR (D_2O) δ: 1.16 (3H, d, $J=6$ Hz), 1.43 and 2.05 (each 1H, br t and br d, $J=12$ Hz), 2.34 and 2.55 (each 1H, each m), 3.63 (3H, m), 3.92 (1H, m), 4.44 (1H, m), 5.97 (1H, m), 6.03 and 6.07 (each 1/2H, each br s), 7.72 (1H, s); ^{13}C NMR (D_2O) δ: 20.6, 34.7, 39.4, 41.3, 62.5, 72.0 (2), 84.2, 87.8, 115.8, 137.9, 150.7, 151.7, 157.8.
   Cyclic 1,N^2-propano 5′-dGMP adduct (1b): 75% yield as a Na salt (as a 3:2 mixture of its diastereomers isolatable on HPLC); IR (KBr): 3420, 1686, 1637 cm^{-1}; UV (MeOH): 275 (sh), 262 nm; Mass (FAB^+): 418 $[M+H]^+$; HR-FAB MS: 418.1123 (mmu: −0.5; calcd for $C_{14}H_{20}N_5O_8P$); ^1H NMR (D_2O) δ: 1.22 (3H, d, $J=6$ Hz), 1.55 and 2.13 (each 1H, br t and br d, $J=12$ Hz), 2.36 and 2.66 (each 1H, each m), 3.66 (1H, m), 3.90 (2H, m), 4.09 (1H, br s), 4.61 (1H, br s), 6.17 (1H, br d, $J=7$ Hz), 6.19 (1H, br s), 7.91 (1H, s); ^{13}C NMR (D_2O) δ: 21.0, 34.8, 39.5, 41.3, 65.5, 72.1, 72.4, 84.1, 86.8, 111.6, 137.9, 151.3, 152.1, 161.0.
   Cyclic 1,N^2-propano 3′-dGMP adduct (1c): 80% yield as a Na salt (as a 1:1 mixture of its diastereomers isolatable on HPLC); IR (KBr): 3398, 1686, 1638 cm^{-1}; UV (MeOH): 275 (sh), 261 nm; Mass (FAB^+): 418 $[M+H]^+$; HR-FAB MS: 418.1136 (mmu: +0.8; calcd for $C_{14}H_{20}N_5O_8P$); ^1H NMR (D_2O) δ: 1.21 (3H, d, $J=6$ Hz), 1.54 and 2.12 (each 1H, br t and br d, $J=12$ Hz), 2.52 and 2.70 (each 1H, each m), 3.65 (1H, m), 3.67 (2H, m), 4.13 (1H, br s), 4.78 (1H, br), 6.15 (1H, br t, $J=5$ Hz), 6.18 (1H, br s), 7.82 (1H, s); ^{13}C NMR (D_2O) δ: 22.3, 36.4, 40.3, 43.0, 64.1, 73.7, 77.5, 86.3, 88.8, 117.8, 140.2, 152.6, 153.5, 159.9.
   Cyclic 1,N^2-propano 5′-GMP adduct (1d): 70% yield as a Na salt (as a 3:7 mixture of its diastereomers isolatable on HPLC); IR (KBr): 3412, 1686, 1637 cm^{-1}; UV (MeOH): 275 (sh), 262 nm; Mass (FAB^+): 434 $[M+H]^+$; HR-FAB MS: 434.1071 (mmu: −0.6; calcd for $C_{14}H_{20}N_5O_9P$); ^1H NMR (D_2O) δ: 1.21 (3H, d, $J=6$ Hz), 1.55 and 2.13 (each 1H, br t and br d, each $J=12$ Hz), 3.70 (2H, m), 3.96 (1H, m), 4.19 (1H, m), 4.34 (1H, br s), 4.58 (1H, m), 5.77 (1H, d, $J=6$ Hz), 6.19 (1H, br s), 7.92 (1H, s).
   Cyclic 1,N^2-propano 3′,5′-cGMP adduct (1e): 93% yield as a Na salt (as a 3:7 mixture of its diastereomers isolatable on HPLC); IR (KBr): 1685 cm^{-1}; Mass (FAB^+): 416 $[M+H]^+$; HR-FAB MS: 416.0978 (mmu: +0.7; calcd for $C_{14}H_{18}N_5O_8P$); ^1H NMR (D_2O) δ: 1.21 (3H, d, $J=6$ Hz), 1.53 and 2.12 (each 1H, br t and br d, $J=12$ Hz), 3.69 (1H, m), 4.13 (2H, m), 4.34 (1H, m), 4.57 (1H, br), 4.89 (1H, m), 5.80 (1H, s, $J=5$ Hz), 6.18 (1H, br s), 7.71 (1H, s); ^{13}C NMR (D_2O) δ: 24.2, 38.2, 44.7, 71.5, 71.6, 75.5, 75.9, 81.4, 96.3, 120.0, 142.7, 154.4, 155.4, 161.8.
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