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Histones Accelerate the Cyclic 1,N^2-Propanoguanine Adduct-Formation of DNA by the Primary Metabolite of Alcohol and Carcinogenic Crotonaldehyde

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Abstract—Chemical modification of 2′-deoxyguanosine and DNA by excessive acetaldehyde and crotonaldehyde were significantly accelerated by the presence of histones, which are nuclear proteins very rich in the basic amino acids such as L-arginine and L-lysine, resulting in the smooth and selective formation of the corresponding cyclic 1,N^2-propanoguanine adducts under physiological conditions. Thus, histones have a very close connection with the genotoxic and carcinogenic effects of these aldehydes.

It is well known that alcohol abusers have highly increased cancer-risks for the upper digestive tract and liver due to their excessive drinking of alcohol.[1–3] The absorbed ethanol is metabolized by two types of dehydrogenases to acetaldehyde (AA) and then to acetic acid. The primary metabolite AA is a highly reactive electrophile and gradually polymerizes in an aqueous solution to give the corresponding dimer (3-hydroxybutanal otherwise known as aldol),[4] trimer (aldoxane: 2,6-dimethyl-1,3-dioxan-4-ol),[5] and tetramer (paraldol: 2-(2-hydroxypropyl)-6-methyl-1,3-dioxan-4-ol).[6] AA and its polymers have been shown to react chemo- and regio-selectively with the exocyclic amino group of the guanine moiety in 2′-deoxyguanosine and DNA leading to the formation of easily hydrolyzable N^2-ethylidene guanines (**1**) as the major product, together with trace amounts of stable adducts such as the N^2-(6-methyl-1,3-dioxan-4-yl)guanine adducts (**2**) and 3-(2-deoxyribos-1-yl)-6-methyl-5,6,7,8-tetrahydropyrimido[1,2-*a*]purin-10(3*H*)-ones (cyclic 1,N^2-propanoguanine adducts: CP–dG adducts) (**3**).[7–9] Among these adducts, the CP–dG adduct (**3**: R = OH) has the capability of making reversible intermolecular cross-links by the formation of carbinolamines, which could dehydrate to the corresponding imines, with the exocyclic amino group of another juxtaposed guanine moiety in the double helical DNA[10] or with the N-terminal amines of peptides.[11] The cyclic adducts easily undergo a preferential oxidative hydrolysis resulting in the formation of the guanine ring-opening products (Fig. 1).[12]

Recently, we documented that in the reactions of excess AA with guanine nucleosides and nucleotides, the dimerization of AA generating 3-hydroxybutanal and 2-butenal (so-called crotonaldehyde: CA), and the condensation of these dimeric products with the exo-cyclic amino group of guanine moiety are significantly accelerated by the presence of a basic amino acid such as L-arginine and L-lysine in the medium to give almost quantitatively the corresponding CP-dG adducts even under mild conditions.[13] Histones are very rich in basic amino acids, such as L-arginine and L-lysine, and combine with the double-helical DNA to play a very important role in the compression of DNA to fit within the nucleus package and in the regulation of the gene-expression.[14] The five major classes of histones, H1, H2A, H3B, H3, and H4, found in all eukaryotic cells differ in molecular weight (between 11,000 and 21,000) and amino acid composition, but L-arginine and L-lysine together make up about one-fourth of the amino acid residues. Therefore, we examined the reactions of

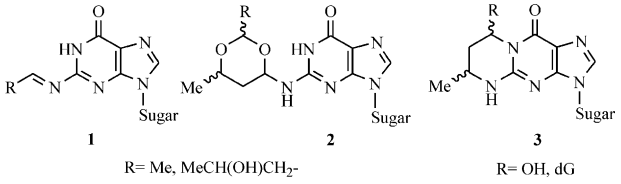

Figure 1. DNA adducts of acetaldehyde or crotonaldehyde.

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Table 1. pH-Dependency of the CP–dG adduct-formation in the reaction of 2′-deoxyguanosine with acetaldehyde (AA) or crotonaldehyde (CA) in the presence of histones

Aldehydes	pH	CP-dG (%)
AA	7.0	14.5
AA	7.5	19.6
AA	8.0	20.7
CA	7.0	36.3
CA	7.5	44.6
CA	8.0	45.9

To a stirred suspension of histones (SIGMA H-6005, Type II-S prepared by extraction in the 1 M NaCl solution) (1.5 mg) in 0.1 M phosphate buffer (pH 7.0, 7.5 or 8.0) (1.0 mL) containing 2′-deoxyguanosine (SIGMA D-7145, 99–100% purity) (2.9 mg, 0.01 mmol) was added acetaldehyde (50 μL, 0.89 mmol) or crotonaldehyde (12.5 μL, 0.15 mmol), and then the stirring continued at 37 °C in a sealed tube for 4 days. The formation of the CP–dG adduct in these reactions were estimated by TLC densitometric analyses according to the reported method.[13]

Table 2. Reactions of calf thymus DNA with acetaldehyde (AA) and crotonaldehyde (CA) in the presence of calf thymus histones or L-arginine

Aldehydes	Pretreatment of DNA	Histones or L-arginine	Relative ratio of CP-dG/10^3 dG
AA	−	—	0.3
AA	−	Histones	2.3
AA	+	Histones	19.5
AA	+	L-Arginine	28.2
CA	−	—	0.6
CA	−	Histones	14.6
CA	+	Histones	103.5
CA	+	L-Arginine	137.2

To a vigorously stirred suspension of DNA (SIGMA D-1501, 16.6A_{260} units/mg) (1.0 mg) (without any treatment or after treatment at 95 °C for 10 min followed by ice-cooling) and calf thymus histones (9 mg) or L-arginine (2 mg, 0.01 mmol) in 0.1 M phosphate buffer (pH 7.5) (1.5 mL) was added an appropriate aldehyde [acetaldehyde (50 μL) or crotonaldehyde (12.5 μL)], and then the stirring continued at 37 °C in a sealed tube for 18 h. LC-ESI-MS analyses were carried out to estimate the CP–dG adduct formation after treatment of the resulting mixtures with 1 N HCl at 70 °C for 1 h, according to the reported method.[15]

2′-deoxyguanosine and calf thymus DNA with excess AA in the presence of calf thymus histones under physiological conditions (37 °C, in pH 7.0–8.0 phosphate buffers). As a result, it was found that the CP–dG adduct formation of 2′-deoxyguanosine and DNA were significantly accelerated by the presence of histones in the medium. The efficiency of the CP–dG adduct formation in these reactions was estimated as a relative ratio versus the unchanged deoxyguanosine (see Tables 1 and 2) by the recently developed LC–ESI-MS method after deglycosidation by virtue of heating the reaction mixtures at 70 °C for 1 h under acidic conditions.[15]

The formation of the CP–dG adducts **3** in the reactions of 2′-deoxyguanosine and DNA with AA was dependent upon the pH of the medium and the DNA–histone complex formation. The employment of a basic buffer and the partially denatured DNA (pretreated at 95 °C for 10 min) resulted in the advanced formation of the CP–dG adducts **3** even under heterogeneous reaction conditions because of the low solubility of the histones in the employed buffer. The present result suggests that the adduct-formation can take place more easily in single-stranded DNA, which could form DNA–histone complexes and physiologically appear in DNA replication and transcriptional events of cells, than the case of the double-stranded DNA which is packaged in the nucleus by the interaction with histones. Thus, the complex formation of DNA with histones is necessary for the effective formation of DNA containing the CP–dG adduct (**3**: R = OH) in the molecule. Analogous results were obtained by the employment of CA in place of AA under similar conditions as shown in Table 2.

Hecht et al. demonstrated that the CP–dG adduct formation is also observed in the reactions of DNA with CA.[16] The aldehydes AA and CA are commonly detected in cigarette smoke, mobile source emissions and other thermal degradation products.[17–20] Therefore, the role of histones in the formation of the CP–dG adducts in DNA is very interesting in connection with the mechanisms for genotoxicity and carcinogenecity of these aldehydes and their precursors such as vinyl acetate and *N*-nitrosopyrrolidine in the human environment.[14,21]

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