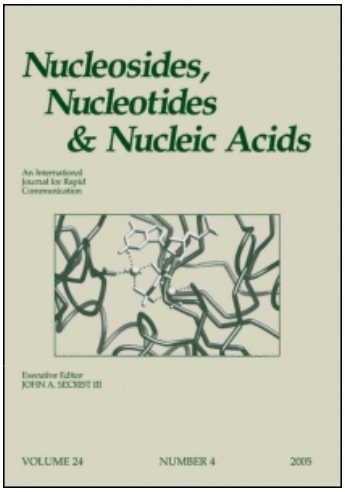

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REACTIONS OF MALONALDEHYDE AND ACETALDEHYDE WITH CALF THYMUS DNA: FORMATION OF CONJUGATE ADDUCTS

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□ *Our previous work has shown that treatment of nucleosides with malonaldehyde simultaneously with acetaldehyde affords stable conjugate adducts. In the present study we demonstrate that conjugate adducts are also formed in calf thymus DNA when incubated with the aldehydes. The adducts were identified in the DNA hydrolysates by their positive ion electrospray MS/MS spectra, by coelution with the 2′-deoxynucleoside standards, and, in the case of adducts exhibiting fluorescent properties, also by LC using a fluorescence detector. In the hydrolysates of double-stranded DNA (ds DNA), two deoxyguanosine and two deoxyadenosine conjugate adducts were detected and in single-stranded DNA (ss DNA) also, the deoxycytidine conjugate adduct was observed. The guanine base was the major target for the malonaldehyde-acetaldehyde conjugates and 2′-deoxyguanosine adducts were produced in ds DNA at levels of 100–500 adducts/10^5 nucleotides (0.7–3 nmol/mg DNA).*

Keywords Malonaldehyde; acetaldehyde; endogenous mutagens; DNA adducts; structural

INTRODUCTION

The biological importance of malonaldehyde and acetaldehyde is derived from their ubiquitous presence in the environment and the continuous exposure of humans due to occupational and life-style factors. Malonaldehyde and acetaldehyde are also naturally occurring products generated endogenously during lipid peroxidation and as the primary metabolite of ethanol, respectively.[1,2] Both aldehydes are highly reactive electrophiles that in reactions with DNA form adducts.[3–5] Formation of adducts can block the normal hydrogen bonding within double-stranded DNA, resulting in miscoding during the DNA synthesis and in initiation of a mutation.

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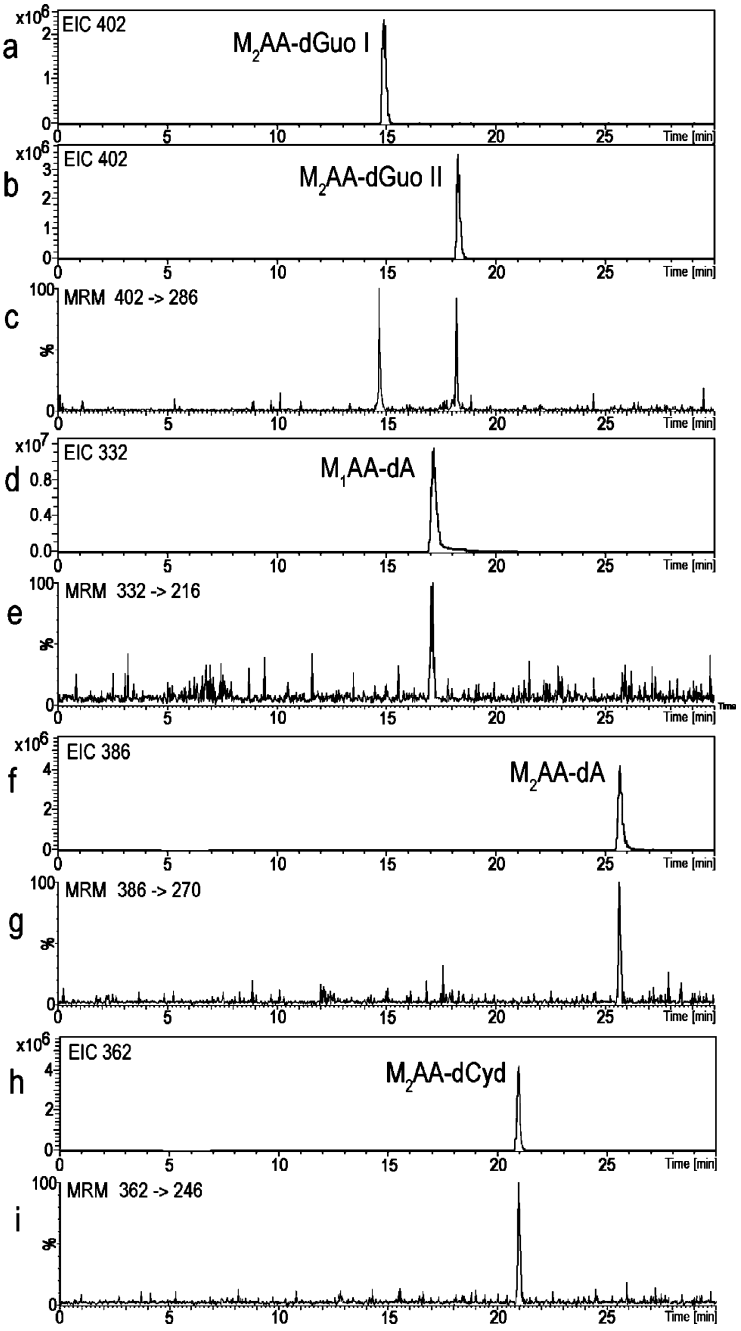


FIGURE 1 Extracted ion chromatograms of the 2′-deoxynucleoside standards (a, b, d, f, h) and MRM chromatograms of the DNA hydrolysate of ds DNA held simultaneously with malonaldehyde and acetaldehyde at 37°C in 0.1 M PB (pH 7.4) for 6 days (c, e, g). MRM chromatogram of the DNA hydrolysate of ss DNA held simultaneously with malonaldehyde and acetaldehyde at 37°C in 0.1 M PB (pH 6.0) for 6 days (i).

Malonaldehyde and acetaldehyde coexist in biological tissues and may undergo an aldol condensation forming highly reactive conjugates.[6] Our previous studies have shown that the conjugates generate cyclic adducts when reacted with nucleosides.[7–9] At physiological conditions only guanosine produced adducts in high yield while other nucleosides underwent reactions at pH 4.6. The purpose of this study was to investigate the formation of conjugate malonaldehyde-acetaldehyde adducts in calf thymus DNA.

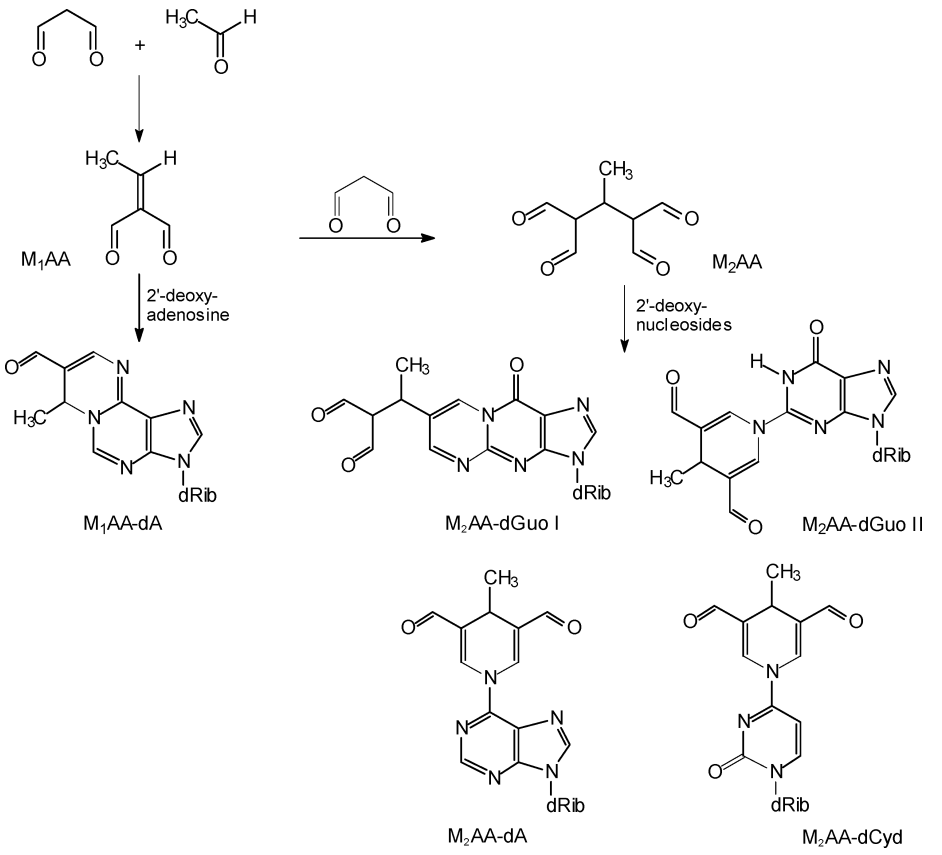
RESULTS AND DISCUSSION

The reactions of malonaldehyde and acetaldehyde with ss and ds calf thymus DNA were performed at 37°C, at pH 6.0 and 7.4 for 6 days. The modified DNA was enzymatically hydrolyzed and subjected to analyses by LC-ESI-MS/MS. Five malonaldehyde-acetaldehyde conjugate adducts were found to be formed in calf thymus DNA: M_2AA-dGuo I, M_2AA-dGuo II, M_2AA-dA, M_1AA-dA, and M_2AA-dCyd. The adducts were identified by their positive ion electrospray MS/MS spectra, by coelution with the 2′-deoxynucleoside standards (Figure 1), and, in the case of adducts exhibiting fluorescent properties (M_2AA-dA, M_1AA-dA and M_2AA-dCyd), also by analysis using LC coupled to a fluorescence detector. The adducts were formed through reactions of the malonaldehyde-acetaldehyde condensation products with the DNA bases (Scheme 1). At physiological pH, the major conjugate DNA adducts were derivatives of the guanine moiety: M_2AA-dGuo I and M_2AA-dGuo II (Table 1).

The identified adducts were formed in higher yields in ss DNA than in ds DNA. This profile of DNA reactivity was detected for the incubation performed at pH 6.0 and at pH 7.4 as well. We do believe that the reason for

TABLE 1 Levels of nucleoside adducts detected in the DNA hydrolysates

Adduct	ds DNA pH 7.4	ds DNA pH 6.0	ss DNA pH 7.4	ss DNA pH 6.0
M_2AA-dGuoI	505 adducts/10^5 nucl (3 nmol/mg DNA)	810 adducts/10^5 nucl (5 nmol/mg DNA)	5971 adducts/10^5 nucl (38 nmol/mg DNA)	1716 adduct/10^5 nucl (10 nmol/mg DNA)
M_2AA-dGuoII	118 adducts/10^5 nucl (0.75 nmol/mg DNA)	81 adducts/10^5 nucl (0.52 nmol/mg DNA)	2216 adducts/10^5 nucl (14 nmol/mg DNA)	515 adducts/10^5 nucl (3 nmol/mg DNA)
M_2AA-dA	14 adducts/10^5 nucl (0.12 nmol/mg DNA)	138 adducts/10^5 nucl (1.18 nmol/mg DNA)	135 adduct/10^5 nucl (1.15 nmol/mg DNA)	1770 adducts/10^5 nucl (15 nmol/mg DNA)
M_1AA-dA	5 adducts/10^5 nucl (0.04 nmol/mg DNA)	42 adducts/10^5 nucl (0.36 nmol/mg DNA)	27 adducts/10^5 nucl (0.23 nmol/mg DNA)	174 adducts/10^5 nucl (1.48 nmol/mg DNA)
M_2AA-dCyd	Not detected	Not detected	Not detected	1786 adducts/10^5 nucl (12 nmol/mg DNA)



SCHEME 1 Formation of conjugate malonaldehyde-acetaldehyde–DNA adducts.

the lower reactivity of ds DNA is that the exocyclic amino groups and N-1 atoms are less accessible due to the hydrogen bonding between the complementary strands. In ss DNA the reactive sites are not hindered and can be easier reached by the aldehydes.

EXPERIMENTAL SECTION

Reaction of Malonaldehyde Plus Acetaldehyde with Calf Thymus DNA

Single-stranded (ss) DNA was prepared by heating the solution of double-stranded (ds) DNA at 100°C for 15 min followed by its rapid cooling on ice. Pure malonaldehyde sodium salt (20 mg, 0.2 mmol) was dissolved in 0.5 mL of 0.1 M phosphate buffer solutions at pH 6.0 and 7.4. Acetaldehyde (10 mg, 0.2 mmol) was added to these solutions and the mixtures were then combined with solutions of ss DNA (2.5 mg) and ds DNA (2.5 mg), in

2.0 mL of 0.1 M phosphate buffer at pH 6.0 and 7.4. The reaction mixtures were stirred at 37°C for 6 days. The modified DNA was further purified and enzymatically hydrolyzed using a procedure described in the literature.[10]

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